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Will Mitterand be free to change?

What happens when one of the most conservative countries in Europe, perhaps even in the world, elects a socialist as a president? Revolution? Or nothing much? Or a mixture of the two? This is what people outside France are asking now that M. François Mitterand has beaten the incumbent president more handsomely in last Sunday's election than even the optimists of the left had been hoping. Frenchmen, however, know the truth.

The new president will be more tightly contained by economic and social constraints than his ardent supporters will be wishing. (M. Mitterand himself, on the other hand, may relish the lack of absolute freedom that he will discover when he takes over in ten days or so.) For the French economy, like others in Western Europe, is balanced on a narrow ledge separating the disasters of inflation and of slump. The temptation of any socialist government to cure unemployment by spending borrowed money is not a cure but a recipe for another kind of disaster. The new president will also find that even his most radical supporters are as firmly wedded to the preservation of familiar institutions as die-hard conservatives. This is why France in the 1980s is so obviously an amalgam of the recent past and the seventeenth century. The plastic bodied automobile on the once-Roman road now lined by poplars is as good a symbol as any.

The influence of the past quarter of a century, since the creation of the Fifth Republic, is nevertheless crucial both for France and for M. Mitterand. With the ending of the neo-Gaullist era, it will be tempting to forget how much has been accomplished. At the beginning of the period, French science and technology were not much to be proud of. There were a few nuclei of industrial innovation, the nuclear energy industry and the new plants for exploitation of natural gas and petroleum in the south and in North Africa, but the telephone system hardly functioned. University research was in the doldrums, chiefly from lack of funds even for teaching the huge numbers of students flocking to the universities. The CNRS was making modest experiments with its scheme for supporting members of staff within university departments, but neither the universities nor the council had begun to appreciate the importance of this device. The university system was hardly equipped to do so, as the upheavals of 1968 demonstrated all too clearly. Yet with the passage of time, French research has prospered. For some reason, imaginative laboratory work has flourished while the government machine has been occupied with the design of frequently artificial programmes built around specified technological goals — the building of an aircraft, or the exploitation of technologies with made-up names, *l'informatique*, for example. Now and again, this peculiarly French system of dual support for research runs into trouble (see page 101). But on the whole, it has functioned better than could have been expected.

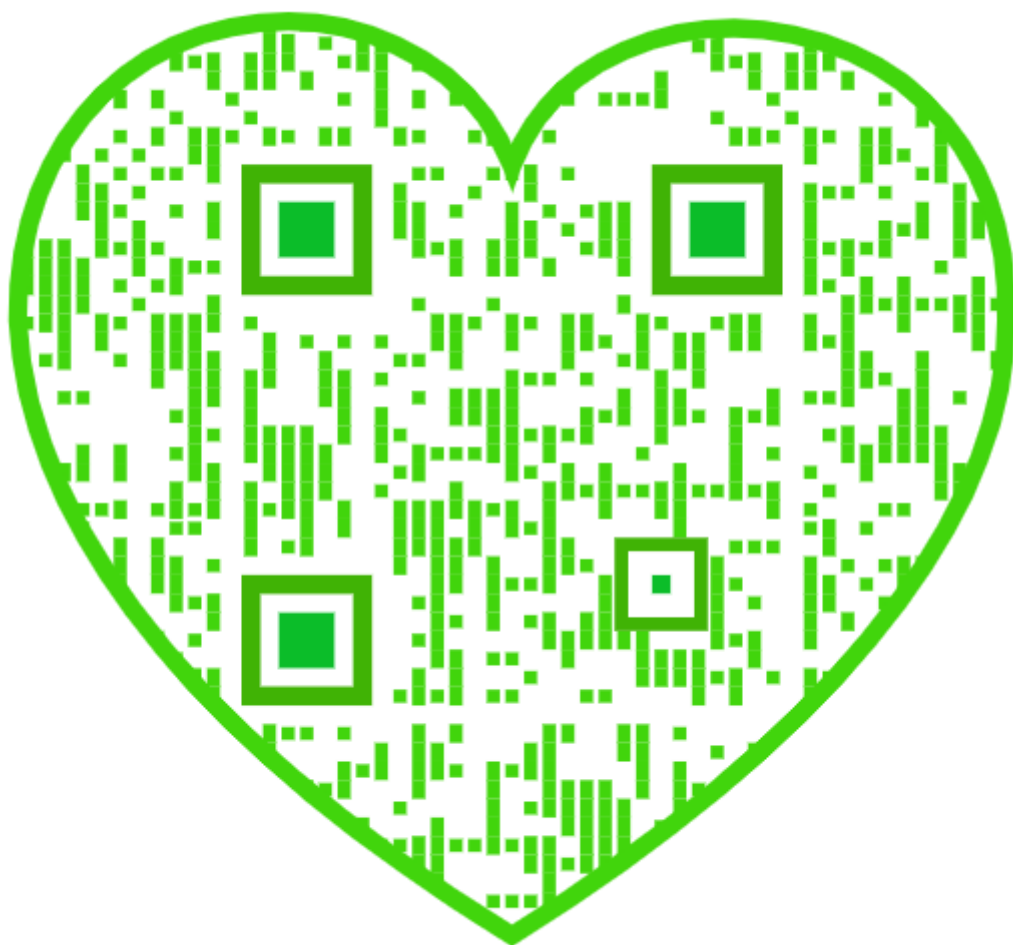
How will M. Mitterand change all this? Nobody at this stage can be sure. Part of the explanation for the new president's success is that he has kept his cards close to his chest during the election campaign. And some of the things he has been saying no doubt owe much to the need to appeal to particular groups of electors. Thus the promise that there will be a "pause" during which the future of the previously ambitious French nuclear power is re-examined may have been designed to win round the ecologists, who captured four per cent of the vote in the first round of the elections. The political cost of reviewing the plans for building nuclear power stations (and for the development of the fast reactor called Super-Phénix) would not be high. Deciding to cut them back would not merely offend a large number of French

workers but would run headlong into conflict with the traditional French conviction that self-sufficiency comes above prudence, economic or otherwise. If the doctrine of self-sufficiency is to be challenged, there are other fields in which the battle could be fought more profitably.

The most obvious opportunity for change is in the not entirely unrelated field of arms control. Since the collapse of the old scheme for a European Defence Force in the mid-1950s, France has been the stumbling block in a long series of arms control negotiations. Once President de Gaulle had accepted the arguments of his generals, General Gallois in particular, that national security required self-sufficiency in nuclear weapons, France has stood aside from sensible arrangements such as the Non-Proliferation Treaty and the Partial Test-Ban. In recent years, French policy has been obviously ambivalent. Thus France has resolutely refused to accede to the Non-Proliferation Treaty while behaving — in dealings with India, for example — as if it had done so. A moderate Gaullist government such as that of President Giscard d'Estaing has inevitably been the prisoner of the true Gaullists to its right, and thus politically prevented from throwing over the foolish doctrines of the 1950s. M. Mitterand will have no such inhibitions. On the contrary, his policies towards the world outside France predicate change. We shall soon find out how resolutely he seeks to amend the French position on nuclear self-sufficiency — and whether in the process he discovers that after a generation, the doctrine of comprehensive military self-sufficiency goes deeper than merely Gaullism.

The prospect that there will now also be a further acceleration of the growth of public spending on research and development is less secure, if only because there are both financial and structural limitations on what the new government can hope realistically to accomplish. French expenditure on research and development, by government and private industry, now amounts to 1.7 per cent of the Gross National Product. M. Mitterand has promised that this will increase by more than a half in the next five years. He has not explained how. And he would find it hard to do so even in the post-election euphoria. Channelling close on an extra one per cent of the national wealth through the government machine, earmarking it for spending on research and development, would be a formidable task if there were a decade in which to do the trick. To attempt it in five years would send inflationary ripples about the place. When he and his advisors start in earnest planning how to implement this promise, they will be driven to conclude that what is true elsewhere is also true in France — that the only basis for spending more money profitably is to begin by improving the quality, and increasing the scale of, university training in science, technology and research. And changing universities is slow work.

The proposal that there should be a minister of cabinet rank with responsibility for research and development is, on the other hand, more than merely sensible. In the circumstances that the new president will inherit, the most immediate danger is that organizations conceived of by bureaucrats and given life by politicians will become so powerful and set in their ways that they are at once wayward and uncontrollable. For all its virtues, the CNRS is in danger of becoming such a law unto itself. The practice of putting university researchers on the payroll, necessary though it may have been a generation ago, now threatens the autonomy of universities and impairs the flexibility of the research enterprise. The first task for the holder of this post (if and when appointed) will be to look again at the relationship between the universities and the rest of publicly supported



research and development. In doing so, he or she will find it necessary to ask whether the condition of the French university system is entirely suited to present needs. In the past seven years of the presidency now ending, much has been done pragmatically to improve the lot of the universities, but very little is written down on paper. The new president seems to have been offering the nearest that France would accept to an American way of administering science. The question still to be answered is whether he will discover, in this connection too, that tradition goes deeper than voting behaviour.

Calculating flop

The British government, always ready to seek to demonstrate that water can be made to run uphill by a sufficient display of determination, has been in trouble for several weeks because it insists on willing that Britain, like the United States, should enjoy the benefits of an indigenous, productive, competitive and profitable mainframe computer manufacturer. The trouble came to a head on Monday, when the chairman and managing director of the company called International Computers Limited (ICL for short) resigned, after the Department of Industry had put a pistol to their heads and asked that they should accept a new way of working. Mr Phillip Chappell, the part-time chairman for just over a year, will go back to being a full-time merchant banker. His managing director, Dr Christopher Wilson, will remain a director of ICL (and will be paid an executive director's salary). Mr Chappell and Dr Wilson are replaced by two outsiders — Mr C. C. F. Laidlaw, vice-chairman of British Petroleum Ltd, as chairman and Mr Robert Wilmot (now managing director of the British end of Texas Instruments) as the new managing director. The government's headhunters have done well; the new management has at least as good a chance of succeeding as the old. But the chances are not high, and it is in the (British) public interest that everybody concerned should acknowledge that to be the case.

ICL has a long distinguished history, going back to the end of the Second World War. That was when people straight out of the design of radar systems settled down to making electronic versions of the old electromechanical calculating "engines". For a time, at least in Britain, it seemed that success exceeded all reasonable expectations. A private company called Ferranti was closely involved with the development of a computing system at the University of Manchester, linked with the names of people such as Thuring and F. C. Williams. The first prototype of this computer was frequently inspected by admiring visitors from other computer companies, many of them from overseas. In due course, when it seemed likely that an electronic computing machine of some kind might sell and make money, the then British government negotiated with Ferranti an arrangement under which there would be a public subsidy to help put this new machine on the market. The amount concerned, just over £1 million, was tiny in comparison with the task. But Ferranti chose to spend this pittance not on the development of a commercial market but on the development of a more advanced and less successful machine.

With the passage of the decades (almost exactly two of them), it became plain that the promise of the immediate post-war period had not been fulfilled. By 1968, the turnover of IBM World Trade (the overseas arm of IBM Inc.) probably exceeded the annual exports of the United Kingdom. Enthused by a vision of a brave new technical world, however, Mr (now Sir) Harold Wilson, then the British Prime Minister, set up an organization called the Industrial Reorganisation Corporation to effect the merger of companies judged too small to compete on their own with the giants overseas. In 1968, Ferranti's computer interests, together with those of the other British manufacturers eager to build mainframe computers, were welded together with the help of public money. The stage was set for the emergence of a giant to compete with IBM *et al.* The snag was that nobody thought, knew or seemed to care what kind of company ICL should be so long as it was big.

This week's announcement that the management must be changed yet again is therefore a sign that water is still steadfastly refusing to flow uphill. The attempts by successive British governments to give ICL an inside track in the supply of computers for the public sector has most often been defeated by the preference of the intended customers for somebody else's machines. ICL shares with the most successful of its overseas competitors, even IBM, the fault of not having judged just when small computers would come into their own. Unlike some of them, it has been too insecure to ride this tide. Now, it seems, ICL is hoping to make up with its flair for distributed computer networks for its failure to make much money on the huge machines that are its pride and joy. British taxpayers in particular will wish it luck. In the next two years, the company will survive with the help of a loan of £270 million from the commercial banks, £200 million of which is underwritten by the government on behalf of the taxpayers.

In retrospect, what went wrong at ICL was not the decision (in 1968) that there should be a unified computer company, nor the British government's repeated reappointment of new managers, but the failure to acknowledge at the outset that in a novel field of technology, it is impossible to design by means of a committee the commercial enterprise that is certain to carry off the prize. The best that can be hoped for is to send several arrows at the target.

The irony in all this is that British government's stake in ICL is now much less than it used to be a year ago. Technically, the company is much more directly under the control of its private shareholders than of the Department of Industry since the government and some other big shareholders sold out to the general public at the top of the market. But ICL will remain in hock to the department so long as the £40 million public loan arranged some years ago remains unpaid. For the whole of that time, British governments will find it necessary to worry about the strategic value of companies such as ICL. Some of them may be able to stomach pulling out of old industries such as shipbuilding, but pulling out of computer manufacturing may seem like turning the back on the future. In ICL's present crisis, all kinds of interests are seeking to persuade the British government of that truth. But it is not a truth at all, of course. Consistently unprofitable companies serve no purpose and should be put to sleep, whatever they happen to be making.

Tokenism again

Public officials, who necessarily spend their waking hours in committee meetings, naturally believe that appearance is reality. For the past several years, but especially since the appearance of the Finniston Report on engineering education eighteen months ago, the British government and the whole company of its officials have been dedicated to the belief that the status of engineering as a profession and of engineers as people must somehow be enhanced. Otherwise, the argument goes, British manufacturing industry will continue to be uncompetitive and will go out of business.

Dr Duncan Davies, until last month Chief Scientist and Engineer at the Department of Industry, and Sir Geoffrey Allen, the chairman of what used to be the Science Research Council, have shown what can be done. Dr Davies has rechristened himself "Chief Engineer and Scientist". Allen's research council, with the help of Queen Elizabeth II, has been renamed the Science and Engineering Research Council. Everybody concerned proclaims that the change of name does not mean a change of function. But engineers (as distinct from scientists) will walk a little taller. And the only cost will be that charged by the suppliers of printed stationery to public agencies and corporations. Nobody seems alarmed that the chief recommendation of the Finniston Report — that there should be an Engineering Council to regulate the profession of engineer — has still not been set up.

At a meeting on Thursday this week, the unions claiming to represent professional engineers will be throwing yet more sand in the works. Tokenism, not the best policy, may be the only one.

Congress will battle against proliferation

Senate split confirmation of Malone

Washington

Democrats in the US Congress are shaping up for what is expected to be a major confrontation with the new Republican Administration over proposals to loosen controls on the export of nuclear technology and materials. Already they have tried, albeit unsuccessfully, to block Mr Reagan's nominee for the position of Chief Nuclear Advisor to the State Department — Mr James Malone — as a warning shot to the Administration that it can expect fierce opposition if it tries to relax existing controls too far or too fast.

Mr Malone had previously been the General Counsel of the Arms Control and Disarmament Agency (ACDA) under President Nixon. Shortly after Mr Reagan's election last November he was appointed to head the transition team for ACDA. And his nomination for the State Department post, officially known as Assistant Secretary for Oceans and International Environmental and Scientific Affairs, has been warmly supported by the conservative Republican, Senator Jesse Helms of North Carolina.

Last week his nomination was formally approved by the Republican-dominated Foreign Relations Committee in the Senate, and confirmation by the full Senate remains a formality. However, five of the fifteen members of the committee voting cast their ballots against Mr Malone, expressing concern that the type of proposals he was supporting for speeding up nuclear exports would increase the risk of the proliferation of nuclear weapons.

Several Democrat members of the Senate committee, including Senator John Glenn of Ohio, a strong supporter of stiff anti-proliferation measures, also raised concerns about possible conflicts of interest. During the Carter Administration Mr Malone has been a member of a Washington law firm, Doub and Muntzing, which includes among its clients groups in Japan who have been seeking permission to export nuclear technology from the United States.

During the committee hearings on his nomination, Mr Malone was reluctant to provide much detail of the extent to which his company had been involved in furthering the goals of such clients. His reluctance provided Mr Glenn with an excuse to hold up his nomination for several weeks, and the committee members unhappy with his appointment are proposing strict procedures to prevent any conflict of interest.

Their main concern, however, is a broader one, namely, that Mr Malone seems keen to dismantle many of the nuclear export controls introduced during the Carter Administration, in particular parts of the Nuclear Non-Proliferation Act of 1978. This act requires countries to accept international safeguards on their nuclear installations before they can receive US-produced nuclear technology or materials, and to obtain US permission before these are transferred to a third party, for example, for the reprocessing of spent fuel.

The transition team report on ACDA suggested that a new office be created within the State Department that would centralize all aspects of nuclear export policy — including the assessment of whether the receiving country was obeying international safeguards, a role at present performed by the Nuclear Regulatory Committee. The nuclear industry, already facing the prospect of a major slump in domestic demand, favours such centralization in order to expedite the licensing of exports; but Senator Glenn and others reply that the Nuclear Regulatory Committee (NRC) was given control of export licensing specifically so that decisions on safeguards would be made on technical rather than political grounds (as happened when NRC refused to allow to the export of nuclear fuel to the Tarapur nuclear plant in India, a decision later overruled by President Carter).

The nuclear industry is hoping for more sympathetic support for its international trade from the new Administration than from its predecessor. The Atomic Industry Forum points out that Secretary of State Alexander Haig has already called nuclear energy "imperative" for Western industrialized nations, and also notes that the appointment of ex-NRC commissioner Richard Kennedy as Under-Secretary of State for Management places "a nuclear supporter in Haig's inner circle" — even though the post has no nuclear responsibility. The industry would welcome a return to the "reliable supplier" doctrine as a device for limiting the misuse of nuclear technology which Mr Malone is expected to promote, but which critics claim has in the past proved ineffective in preventing nuclear exports claimed necessary for energy programmes being used for military purposes.

Ironically, the major threat may come from a very different direction, the Administration's desire to reduce public spending substantially. Among the agencies expecting major budget cuts is the Export-Import Bank, which provides loans to US companies to subsidize their exports, particularly to Third World countries. Virtually all US nuclear exports have been paid for with loans from this bank. If the source of the loans dries up — as seems possible — the nuclear industry will find itself facing a new set of hurdles to cross.

David Dickson

Science Adviser appointment revived

Washington

The appointment of a Presidential Science Advisor, on the back burner for most of the time since Inauguration Day, has become a live issue again. In the past few days, the name of a potential candidate for the job has been circulating. Earlier this week, it seemed certain that the new man would be formally nominated before the end of May.

The newest candidate is Dr George A. Keyworth, 41-year-old head of the physics division at the Los Alamos National Laboratory in New Mexico, where he has worked for the past 13 years in nuclear weapons research, laser fusion and basic nuclear physics.

Dr Keyworth has already visited Washington to discuss the job with White House officials, but said last week that he has not formally been offered the position — although the offer was widely expected in Washington. He holds a bachelor's degree from Yale University in 1963, and a PhD in physics from Duke University in 1968.

Dr Keyworth is largely unknown in the scientific community; he seems to have had little experience in Washington, although he has served on the Department of Energy's Fusion Data Committee. Dr

Keyworth's name is said to have been put forward by the domestic policy staff within the White House, and he is said to have the enthusiastic support of Dr Edward Teller, one of Mr Reagan's advisers when he was governor of California.

The Reagan Administration has apparently had considerable difficulty in finding a candidate prepared to accept the post of science adviser and director of the Office of Science and Technology Policy, partly because of rumours that the role of the office is likely to be downgraded from its prominence during the Carter Administration under Dr Frank Press. Dr Keyworth says that he is in complete agreement with the new Administration's attitude towards science, and attempts to restore "American pre-eminence in industrial technology".

Dr Nunzio J. Palladino, dean of the College of Engineering at Pennsylvania State University, is expected to be named by Mr Reagan as the new chairman of the Nuclear Regulatory Commission. Dr Palladino worked as an engineer with the Westinghouse Electric Company in Philadelphia before joining the National Laboratory at Oak Ridge.

David Dickson

French presidential election

Good for science?

Champagne corks were popping in science departments throughout France on Monday at the news that François Mitterand, socialist party leader, was to be France's next President. Not that most French scientists are socialists; rather, that science will take a leap forward in France if Mitterand sticks to his announced policies.

Mitterand has promised a "national council for science and culture", reporting to the president, and has announced that spending on research and development in France should reach 2.5 per cent of gross national product by 1985, compared with the present 1.8 per cent. Mitterand's goal could only be reached with high government spending, for French industry is notably niggardly in its provision for science; Giscard had a more cautious target of 2.3 per cent by 1988.

The socialist leader has also said he will raise the rank of the minister of science to full cabinet level, so the minister will participate in all major policy decisions. (The current incumbent, Pierre Aigrain, attends cabinet only when matters involving science are under discussion.) Two names are being tipped for the job; François Gros, a biochemist at the Institut Pasteur, who has been active among scientists on Mitterand's behalf; and the career politician Jean-Pierre Chevènement, a member of the chamber of deputies who has taken an interest in scientific affairs. The high level of the post may favour the politician.

Mitterand will also set up a parliamentary special committee for technology assessment, something previously unheard of in France, where technological decisions have been reached in an entirely autocratic manner. The new president is also cool on nuclear power, so France's nuclear lead might be expected to slip unless national pride erodes the president's doubts. He has said he will call for a "pause" in nuclear construction after the reactors now under order, and the fast breeder Super-Phénix, are completed, in order to review the programme.

Robert Walgate

French oceanography

Pulling together

The outgoing French government completed the last leg of its reorganization of oceanographic research last month with the creation of a joint interdisciplinary programme between CNRS, which is the main funding body for scientific research, and the Ministry of Universities. The new programme is expected to be in full operation early next year. The reorganization, which began at the end of 1979 when an informal Comité d'Océanologie was set up to advise government, reflects the political importance in

France of technologies for exploiting the mineral and biological wealth of the oceans.

This attempt to coordinate research supported by CNRS and the Ministry of Universities through the new Programme Interdisciplinaire de Recherche Océanographique (PIRO) is unusual in French research administration, for with the growth of support for oceanography since the Second World War, two disparate research programmes have grown up within the universities and the CNRS laboratories. Although similar difficulties have arisen in other fields, oceanographic research has been particularly affected, according to the director of PIRO, Roger Chesselet.

PIRO's task of coordination will fall to an eighteen-member scientific committee with an annual budget of 11 million FF, the Ministry of Universities and CNRS each contributing half. The budget, which will be in addition to the 185 million FF already spent on oceanographic research,

will be used to run and buy equipment for six small research vessels, and to foster collaboration between several research groups working on different aspects of the same problem. PIRO, whose members are CNRS administrators and oceanographers nominated by CNRS and the Ministry of Universities, will be keen to support fundamental research in physical and biological oceanography, leaving mineral exploration and the application of new technologies to the well-established Centre National pour l'Exploitation des Océans (CNEXO). A small amount of the extra 11 million FF will, however, be spent on research equipment for use by CNRS and university staff on board CNEXO's large, ocean-going research vessels.

The setting up of PIRO follows the definition of three categories of research: research with short-term application supported by the departments of industry, transport and environment; research with medium- and long-term application supported by CNEXO; and fundamental research, the responsibility of the Ministry of Universities.

Under the new arrangements, the different organizations supporting oceanography will report to the Comité d'Océanologie. France is also hoping to collaborate internationally, especially with developing countries, on pollution problems and the exploitation of ocean resources when the Law of the Sea becomes operational.

Judy Redfearn

Bazan protests

The academic research community in Argentina has been disconcerted by the precipitate sacking of Professor Nicolas Bazan from his posts as Director of the Institute of Biochemical Research at Bahia Blanca and as Professor of Biological Chemistry at the University of the South. Professor Bazan (38) heard of his dismissal only when he returned to Argentina from the United States early in March, but it is held to have taken effect from the end of 1980.

The most puzzling aspect of this development is that Professor Bazan has insisted that he has no connections with political organizations deemed subversive by the military regime. One explanation is that his success has made him the target for influential jealousy, another that his dismissal is intended as a reminder that the influence of the military persists in the universities of Argentina.

Dr Bazan is a neurochemist who has, in the past few months, been able to demonstrate that in rat models of epilepsy, arachidonic acid and other precursors of prostaglandins are released into the brain in large amounts. Professor Bazan had been planning an investigation of the presence of arachidonic acid in the cerebrospinal fluid of human patients suffering from epilepsy, on the assumption that the underlying cause is a membrane deficiency.

Since his dismissal, Professor Bazan has insisted that he will fight for reinstatement. He has been supported by the staff of his institute, which sent a letter of protest to the President of Argentina on 21 March. The Argentinian press has also covered Bazan's dismissal sympathetically.

UK university finance

Truth looms

British universities should know more clearly next week what the future holds. For then the University Grants Committee will put out a circular spelling out the basis on which recurrent grants will be distributed to individual universities in the next few years. This first circular will be followed in June by more detailed information about the proposed division of the recurrent grant for 1981-82 among individual universities.

The committee has been forced to this two-stage approach by the need for detailed consultation with the Department of Education and Science about ways of sweetening what must certainly be a bitter pill. Originally it had been intended that final allocations would be announced this month. One of the concessions wrung from the government is likely to be an assurance that recurrent funds for the universities will be held for the next three years at the levels suggested in the white paper on public expenditure published in March.

British universities may thus know where they stand more clearly than in recent years, when government support has been determined one year at a time. The promised grants will, in the government's opinion, nevertheless require many universities "to review the range and

nature of their contribution."

Next week's letter from the grants committee is expected not to go so far as to suggest that some universities should close. The emphasis, instead, will be on financial economies within existing institutions, with the recommendation that universities should think of closing departments which, for one reason or another, do not meet tests of efficiency and effectiveness. Universities and those who work in them will be especially interested in whatever arrangements the committee suggests for dispensing with unwanted members of teaching staffs.

Dr Edward Parkes, chairman of the University Grants Committee, has, however, made it clear in his evidence to a parliamentary committee that the impending cuts will not be made uniformly across the board. Universities will also be affected differently by the still uncertain enrolment of overseas students for the coming academic year. In its *Statistical Bulletin* for April, the Department of Education and Science shows that overseas student numbers declined by 7 per cent between 1979-80 and 1980-81.

Enrolments between the same two years declined by 10 per cent. Although the universities as a whole have more successfully maintained overseas student numbers than other institutions of higher education, some among them are in serious trouble, while all fear that new enrolments next October will decline further.

One part of the government's package of support for the universities has, however, been decided. The equipment grant for British universities will be increased by about 11 per cent to £76.7 million for the academic year 1981-82.

Hungarian research

Cost of elitism

Budapest

Budapest's Eotvos Lorand University is to have a new science building — at a time when the Hungarian government is drastically reducing spending in virtually every sector of the economy.

This is not the first time that the university has been promised such a building, which would unite its 44 small science "chairs" under one roof into four large departments — mathematics and physics, chemistry, life sciences and Earth sciences. Yet, in spite of the economic climate, the academics concerned are confident that this time the plans will come to fruition, because the reorganization can be justified as cost-effective. With all laboratories in close proximity, it will be possible for the various departments to share expensive equipment.

More significant in precipitating the decision, however, was the public debate on the role of the university, begun in May 1979 by the journal *Magyar Tudomány*, in which the reorganization of universities

into large departments was favoured.

One of the government's aims is to break the tradition of academic elitism. Young scientists are reluctant to leave cosy academic niches in research institutes to enter the harsher world of industry and the universities. Although the reorganization will be welcome, any real moves to strengthen the role of the universities in research would first have to deal with one of the major grievances of Hungarian scientists, the difficulty of purchasing equipment and reagents from abroad.

Because of the centralization of foreign trade, such purchases have to go through a single import-export company, which means a delay of at least six months for every order. Hard currency is not the only problem — the same delay occurs with purchases from the Comecon bloc. Only in research projects with a government priority rating — such as work on genetic engineering done under contract with the chemical industry — can the purchasing process be accelerated.

Whenever this subject is raised with officialdom, scientists say, the response is always sympathetic — but without visible result. Until the matter is resolved, however, young scientists will have one more reason for seeking a long-term post in one of the Academy of Science's numerous research institutes. For the academy has its own purchasing agency, "Akadimport", and although the delays involved are still of the order of two months, this still gives such researchers a considerable edge.

Vera Rich

Satellite-linked computers

Network planned

Several different groups in Britain are cooperating in an experiment to link computers via satellite. The idea is to demonstrate to home and overseas customers how British technology can help industry and research institutions with their communications problems. Project Universe, as the experiment is called, is being supported to the tune of £3 million for three years by the Department of Industry, the Science and Engineering Research Council, British Telecom and two private companies, GEC-Marconi and the software house Logica.

The experiment is mainly to demonstrate how groups of users such as universities and companies with offices in different centres can communicate with each other through networks of computers linked by land and satellite. Six laboratories will house rings of computers, based on a network designed at the University of Cambridge, and will be equipped with an earth-station consisting of a 3-m diameter dish a 14-GHz radio transmitter and an 11-GHz receiver. Communications within a laboratory will be via the land system and between laboratories via one channel of the European Space Agency's Orbital Test

Satellite. Each computer will be able to communicate with any other in the system initially at a rate of 1 Megabit per second.

The Department of Industry, which is providing the largest sum of money, hopes that the project will help develop technology and demonstrate uses for the network.

The two major demonstrations of the network will be in transmitting large volumes of scientific and industrial data between centres. The Rutherford Laboratory is particularly keen to use the network to transmit mask design for large-scale integrated circuits, developed with its electron beam lithography machine, to universities and industry.

The project is expected to be in operation early next year. It will run for two years throughout the rest of the life of the Orbital Test Satellite.

Judy Redfearn

Research management

Audits go on

Despite the tangle of legal threats, and claim and counter-claim over their previous — and still unpublished — work on Sir Bernard Lovell's radioastronomy at Jodrell Bank, a group of the Science Policy Research Unit (SPRU) at the University of Sussex has been given the go-ahead for its next study: CERN at Geneva.

The group is headed by Keith Pavitt, acting deputy director of SPRU, with principal researchers John Irvine and Ben Martin, who have developed what they call the method of "converging partial indicators" to assess the relative merits of scientific research groups.

Inevitably, this is dangerous territory, normally confined to the secrecy of peer review panels and referees' reports, and the Sussex group's serious attempt to arrive at objective criteria raised hackles as soon as it was applied to real cases, which included a comparison of radioastronomy groups in Britain and abroad and a study of British optical astronomy.

All these studies are now essentially complete, but none has been officially published — largely due to the Sussex group's procedure of checking its figures with the relevant laboratories first. Predictably, laboratories whose work is implicitly criticized object to either the figures or the conclusions.

The SPRU group, however, has been unable to consider such questions because at an early stage it was denied access to research council files under the "40 year rule", which controls the availability of government documents — even though the research councils are not government departments. It therefore could not assess the conditions under which one group, say, received a major grant, while another did not.

Nevertheless, what counts in the end is results, and the SPRU group claims to have a method which gives an objective assess-

ment of those results by using the relevant research community's own judgements of scientific value, both directly (through confidential questionnaires) and indirectly (through publication rates and citation frequencies).

A preliminary version of the radio-astronomy study was read last year at a closed session of the Organization for Economic Cooperation and Development, and was very well received; but the wide circulation of that paper, particularly in the Netherlands and West Germany, whose own radio telescopes were also assessed (not all favourably) led to recrimination from Jodrell Bank, which did not do so well in the study. And now North-Holland, the publisher of *Research Policy*, in which the final paper was to appear, is said to be refusing to publish the radioastronomy work.

Moreover, the originator of the SPRU study, Sussex physicist Dr Norman Dombey, wishes to have no part in such publication, and says he disagrees completely with the work, mostly done by Martin and Irvine, and its conclusions. Dombey, although a high energy physicist, will not be a member of the group for its study of CERN, and argued with the Social Science Research Council that the CERN work should not be funded. However, after nearly a year's deliberations, the council has given the group a grant for 15 months' work on CERN and its future project LEP, but has insisted this time on extremely strict rules on confidentiality.

Robert Walgate

Indian government policy

Committed to science

New Delhi

The poor coordination between Indian scientists and politicians should improve following long-awaited decisions announced by Mrs Gandhi recently. Mrs Gandhi herself is to preside over a nine-member Cabinet Committee on Science and Technology, dealing with broad policy matters, and two more science committees have also been formed.

India's worsening position on the energy front and a massive bill for oil imports have led to the formation of a six-member Alternative Energy Commission, along the lines of the Atomic Energy Commission, to be chaired by Professor M. G. K. Menon, Secretary of the Department of Science and Technology. The third committee, the Science Advisory Committee to the Cabinet, consists of 20 eminent Indian scientists from all the major disciplines. Dr M. S. Swaminathan heads this body which is to advise the cabinet on science and technology policy.

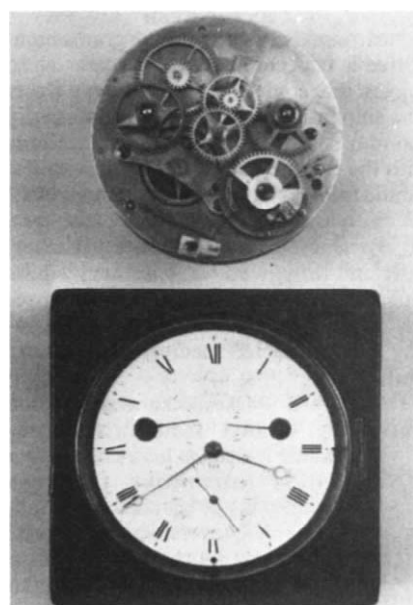
The Cabinet Committee on Science and Technology will have an important role in deciding how to spend the science and technology budget of more than 30,000 million rupees (£1,667 million) allotted in

Arnold at Christie's

An 8-day marine timekeeper which is being offered for sale on 3 June at Christie's London auction rooms is thought to be a prototype for the modern marine chronometer, and it has been suggested that the spring detent which it employs could be the earliest ever, pre-dating the putative "Earnshaw" pattern by several years. Made by John Arnold in London around 1775, the design of this un-numbered chronometer throws light on the many problems which confronted Arnold in his efforts to construct a timekeeper which would be accurate and reliable and could also be made in quantity at a relatively low price.

His chronometer (a term he himself coined) was designed to keep the same rate of going in every position and it incorporates a mechanism to compensate for changes in temperature. It has a three-armed uncut brass balance 45.2 mm in diameter. Regulation is by flat spiral balance spring, regulated by curb pins carried on a bi-metallic compensation curb, concentric with the balance pivot. The convex enamelled dial bears the signature "Arnold, London". His earliest dials were enamelled, but once commercial production was started, he changed over to silvered dials. The box, 6½ inches square and 3½ inches deep, is of mahogany.

Arnold first started working on marine timekeepers in 1786, about nine years after John Harrison — the carpenter turned clockmaker — had submitted his No.4 timekeeper to the Board of Longitude. This was a body which had been set up by the British government and which in 1714 offered a prize of £20,000 for a machine which would enable longitude at sea to be determined to a specified degree of accuracy. Harrison richly deserved the prize but the board shilly-shallied for many years, and it was only towards the end of his life that he received the final instalment of the award.



Had the Board of Longitude a free choice between the Harrison and Arnold timekeepers, the Arnold might well have been chosen on the grounds that it was less complicated and consequently easier to produce. Arnold made chronometers for the Admiralty at 60 guineas a piece, much cheaper than Harrison's which took about 3 years to make.

Arnold deserves much of the credit for introducing into England the manufacture of chronometers on a large scale.

As for the price likely to be reached when the chronometer is auctioned, Christie's are not willing to speculate. But if the device's history can be authenticated it could fetch anything between £20,000 and £100,000. **Arthur Frank**

Mr Arthur Frank, the now-retired owner of a Glasgow-based optical business, is a well known collector of optical and scientific instruments. Much of his collection, which includes early telescopes, cameras and eyeglasses, is now distributed among several British museums. Mr Frank will be contributing occasional articles when important scientific instruments change hands or come onto the auction markets.

the country's sixth five-year development plan. Main areas of interest will be in life sciences, oceanography, alternative energy sources and bioengineering as applied to pharmaceuticals. **Naresh Sahajpal**

● **Energy alternatives:** The Indian government has given fresh impetus to the development of new and renewable sources of energy. The key point of the plan is reduced dependence on imported oil. The government is investing in systems for utilization of energy from the Sun, wind and biomass.

In photovoltaics, the public sector company, Central Electronics Limited (CEL) of Sahibabad, has begun bench-scale manufacture of single crystal silicon solar cells and panels with capacities of up to 10kW per year. Solar modules developed by CEL are already being used in

powering a light beacon at Dwarka port, pumping water for a whole village in Rajasthan and lighting a 20-bed hospital in Ladhak. Solar water heating systems are being installed in many hotels, and a textile mill in Ahmedabad is already meeting its hot water requirements through a large solar heater.

A 120 million rupee (£7 million) project has been drawn up for developing low cost solar-grade silicon material and cheap fabrication techniques and for improving the efficacy of solar cells and solar panels.

A national wind energy centre is being set up at Allahabad Polytechnic to develop a low-cost windmill. A cold storage plant utilizing geothermal energy is being set up in Himachal Pradesh. And a 600 MW tidal power station is planned in the Gulf of Kutch in Gujarat. **Zaka Imam**

Third World development UN funds falter

Washington

Frustrated in the United Nations by lack of progress in following up proposals from the Conference on Science and Technology for Development (UNCSTD), held in Vienna in August 1979, a group of developing countries has organized a top-level mission to try to persuade the oil-rich nations that they should be doing more to support technological efforts in the Third World.

In an initiative originating from government officials from Tunisia, Mexico, Pakistan and Guinea, a delegation made up of ministers or other top officials from ten developing countries will visit several major oil-producing countries at the beginning of next month to discuss ways of supporting technical assistance programmes. The countries to be visited include Saudi Arabia, Kuwait, the United Arab Emirates, Nigeria and Venezuela.

The delegation will then move to Paris, where it will report to the Secretary-General of the Organization for Economic Co-operation and Development (OECD). Several OECD countries, in particular the Scandinavians, have already expressed enthusiastic support for the so-called "Tunis initiative", which they see as avoiding some of the road-blocks faced by classical negotiating patterns on North/South economic issues.

Eventually it is hoped that a package can be put together in a form likely to be endorsed by the planned summit meeting between industrial and developing countries in Mexico City in October. President Ronald Reagan has recently agreed to attend the meeting (partly, it is rumoured, because President Lopez Portillo of Mexico has agreed not to invite Cuba's Fidel Castro).

The package could contain a commitment from oil-producing and developed countries to support technological development in the Third World by several hundred million dollars a year by the mid-1980s. The aim is to find a way of using the large cash surpluses being built up by the oil-producing nations to catalyse development efforts, without running into the political tensions which have polarized discussions between the Group of 77 and the industrialized nations within the United Nations.

In the event, the Interim Fund for Science and Technology, set up at the Vienna conference with an initial goal of raising \$250 million to cover two years of operation, has fallen far short of its target. Less than \$60 million has been received in pledges — and only about a third of this is thought to have been handed over. The interim fund is the responsibility of the United Nations Development Programme, and officials are working closely with the Tunisian government and other members

of the so-called group of 10 — in addition to the four countries listed above, the other members are Peru, Senegal, Sudan, Kenya, Jordan and Sri Lanka — to find other ways of financing the projects that they have been assessing.

Although financial aid will be high on the agenda of next month's discussion with the oil-producing nations, equal emphasis is being placed on the need to support science and technology as an integral component of economic development in Third World nations. Even the oil-producing nations themselves are putting increasing emphasis on their need for technical assistance in building an economy which is not totally dependent on oil exports.

Officials from the United Nations Development Programme are also trying to link the efforts which have already been made under the interim fund to any plans emerging from the UN Conference on New and Renewable Sources of Energy, due to take place in Nairobi in August, for encouraging research on new energy sources. They argue that it would avoid

unnecessary duplication if any new research initiatives from the Nairobi conference were under the same umbrella.

A second list of projects, totalling \$11.2 million, has recently been approved for funding under the interim fund. These include \$1.3 million to Brazil aimed at improving carbon fibre technology; \$500,000 to Ethiopia for the popularization of science and technology; \$670,000 to establish a unit for research on cellulose products in the Sudan; \$370,000 for "interregional" courses in Bangladesh and Ghana on the application of modern techniques in physics to development (one on monsoon dynamics, the other on solid state physics); and \$30,000 towards a symposium in Ghana on the state of biology in Africa, being organized under the auspices of the International Council of Scientific Unions. Funding has already been offered for the initial stages of each of these projects, although whether the totals are reached depends on how many countries make good their pledges to the interim fund.

David Dickson

Mary Lindley

Mary Lindley, an assistant editor of *Nature* since 1966, died last Thursday, 7 May, after some months of illness. She was the longest-serving member of the present staff of *Nature*, and during her service with the journal had successively been responsible for most of the journal's separable functions. She joined the staff immediately after graduation from the University of Oxford, where she had been trained at Somerville College as a biologist with inclinations towards botany.

Mary Lindley suffered throughout her life from the consequences of restricted growth. Her method of dealing with this handicap was to behave as if it did not exist. In this spirit she learned to type, to drive a motor car, to live on her own and to travel widely. Between 1972 and 1975 she worked in the Washington office of *Nature*, handling the flow of scientific manuscripts through that office. When she died, Mary Lindley was the chairman of the Association for Research into

Restricted Growth, an organization whose formation she had helped to stimulate.

As a colleague, one of Mary Lindley's memorable attributes was her sardonic wit. Always economical with words, she could in half a sentence puncture an overblown argument — or make a joke. For much of her time on *Nature*, she was the chief defendant of the view that whole organisms have a place in biological studies.

She appeared to be indefatigable. To avoid the worst of the London traffic, it was until very recently her habit to set off in her tiny motor car before the rush-hour began. She would leave the office late, not only to avoid the evening rush-hour but because (she would insist) there was still work to do. Her greatest achievement was that her dealings with those whom she met in the course of her work were never compromised by her handicap; to them, she was merely a very sharp lady who happened to be small.

As an editor, Mary Lindley was the most flexible of people. In her time, she had been responsible for the Book Reviews and the News and Views sections of the journal. For more than a year before she died, she had coordinated, edited and written much of the output of the *Nature*-Times News Service, which appears in the London *Times*. A few days before she died, cheered by her release from an iron lung and apparently indifferent to the discomforts of a tracheotomy, she asked for a typewriter to be taken to St Thomas's Hospital so that she could begin to write again.

Very few knew of her mounting frustration in the past year about her deteriorating health. Her courage was, however, evident to all.



CORRESPONDENCE

Progress in Crete

SIR — The commentary on the current conditions in higher education and research in Greece by Mr E.M. Pantelouris, which appeared in your issue of 1/8 January, came to our attention with some delay. It is, however, instructive to examine the case of the University of Crete as an example of the roadblocks encountered in the actual implementation of innovative ideas and the measure of success that may be expected. This will also demonstrate, we hope, that some of the conclusions of Mr Pantelouris with regard to the University of Crete are not necessarily true.

The operations of the University of Crete started in the academic year 1977–78 with the Department of Mathematics of the School of Sciences at Heraklion and the School of Humanities at Rethymnon. The Department of Physics was added in 1978–79. During this formative phase, instruction was carried out by temporary staff hired on an annual basis and the university was housed in provisional quarters provided in high school buildings.

In spite of these shortcomings, the progress accomplished during the first three years of operation through the efforts of the Provisional Administrative Committee and the enthusiasm of the temporary staff has been remarkable. Some of the major accomplishments include: (1) The organization of a library which already contains about 100,000 volumes in the humanities and 20,000 volumes in sciences. (2) The construction of provisional housing facilities for the schools of science and medicine which are expected to be ready for the academic year 1981–82. (3) The development of a flexible modern curriculum which has been already used successfully. And (4), the organization of student laboratories and the procurement of basic research equipment for the School of Sciences.

Clearly, however, the consolidation of these achievements and the future progress of the university depends critically, at this stage, on the recruitment of a high quality permanent faculty. The Administrative Committee has pushed from the beginning for a departmental structure, in contrast to the chair system, with a high professor to student ratio and a faculty with three professional ranks. The initial appointments will be made by faculty selection committees nominated by the Administrative Committee and consisting of professors from universities in Greece and abroad. These are new and unprecedented features for the Greek university system and it is not surprising that the legislation required for their implementation was time consuming.

This work is now complete and a call for candidacies for 24 positions in the School of Sciences and 14 positions in the School of Humanities has been publicized (see *Nature* classified columns of 29 January). The faculty selection committees have been nominated and the first appointments are expected to be made by late autumn this year. These, of course, are only the first steps in consolidating the structure of the new university and much persistent and enlightened effort will be required especially by the first members of the faculty. The course of the campaign for reforms in the system of higher education in Greece has demonstrated so far that while a diagnosis of flaws and deficiencies is common

knowledge, the planning and implementation of effective steps for lasting changes is an agonizing procedure, replete with frustrations but highly rewarding when successful. We hope that the University of Crete will play a significant role in this by setting an example of a high quality modern institution of higher education and research.

A. KOSTIKAS
(Member of Administrative Committee)
Crete University, Greece

Xeroderma list

SIR — The issues raised in Cairns's article "The origin of human cancers"¹ and subsequent comments² highlight the importance of studies of rare human disorders as models for human carcinogenesis. In order to gain quantitative information on such questions as the frequency of cutaneous and internal neoplasms in patients with defective DNA repair, we are establishing a registry of xeroderma pigmentosum patients. We welcome correspondence from physicians and scientists who are aware of American patients with xeroderma pigmentosum:

Xeroderma Pigmentosum National Registry,
c/o Department of Pathology,
Medical Science Building, Room C520,
CMDNJ-New Jersey Medical School,
100 Bergen Street,
Newark, New Jersey 07103, USA

ALAN D. ANDREWS
JAMES L. GERMAN III
W. CLARK LAMBERT

Columbia University, The New York
Blood Center, NIH
and CMDNJ-The New Jersey
Medical Center, USA

1. Cairns, J. *Nature* **289**, 353–357 (1981).
2. Trosko, J. *Nature* **290**, 356 (1981).

Be nice to NATO

SIR — Mr Roger R.C. New has recently argued in these columns against publicizing the NATO Advanced Study Institutes in *Nature* on the grounds that it implies approval for "the increasing military orientation of our society" (*Nature* 23 April, p.624). I take precisely the opposite point of view (neither of us questions the scientific and pedagogical value of these institutes).

Surely lasting peace can only be established on this planet if a framework of trans-national judicial, political and economic institutions can be developed to cope with the problems plaguing all mankind. In my view the construction of such institutions must begin with those nations sharing a common democratic tradition — basically those encompassed by NATO and/or OECD. NATO has always been intended as more than a military alliance; I note Article 9 of the Treaty, establishing the "North Atlantic Council" for political consultation, and the meetings of the NATO parliamentarians.

Expansion and encouragement of multinational intellectual, economic and ultimately political ties among the citizens of the world's democracies must be urgently pursued, and the NATO Advanced Study Institutes play a small but positive role in such developments.

WILLIAM M. IRVINE
Chalmers University of Technology,
Onsala Space Observatory, Sweden

Computer algebra

SIR — I was delighted to see your recent article on "Algebra made mechanical" (*Nature* 19 March p.198). These symbolic math systems will surely revolutionize scientific computation. However, there is one minor correction to the article: muMATH was coauthored by Albert D. Rich and myself at The Soft Warehouse. Also, to redirect the deluge of enquiries, I suggest that they be sent directly to the distributor: Microsoft Consumer Products at 400 108th Ave. N.E., Suite 200, Bellevue, Washington 98004, USA.

DAVID R. STOUTEMYER
The Soft Warehouse,
Hawaii, USA

Confusing script

SIR — In the debate stimulated by Halstead's¹ criticisms of the Natural History Museum's exhibit on human evolution, there are two main issues which only partly overlap. First, what cladism entails, and what various authors think about it; and second whether the museum's exhibit presents a fair picture of human evolution, as it is known to science.

Those who disagree with Halstead about the former would do well not to prejudge his assessment of the latter, without seeing the exhibit. B.A. Wood's² observations that some early hominids have been seriously misassigned should be noted. So far the archaeological side to the exhibit has passed without comment, but one particular example, in which archaeology and physical anthropology are brought together, speaks volumes for the general tone of the exhibit.

First, it is suggested, reasonably enough, that neanderthals have characteristics not shared by modern man, and vice versa. Then the script continues

"But because the neanderthals and modern man share these two important characteristics — an average brain size of 1,330 ml — burial of the dead . . . they can be grouped together in the same species — *Homo sapiens*".

This confused conjunction of biometrical and cultural criteria is so weird that little more need be said. But other oddities abound. We are told that we are apes, without any reference to the pattern of characteristics which distinguish us as hominids. Then, the whole, vital, subject of chronology is almost totally omitted.

It seems unlikely that the exhibit represents "official" doctrine of the museum concerning cladism. It represents a good visual show, in which unfortunately "educationalese" has taken over to the extent that the children and other visitors who much enjoy the exhibit are seriously and unnecessarily misled by gross oversimplifications. This is a great pity, for in many ways meticulous care has been taken in the preparation of the exhibit. The desire to simplify in order to spread knowledge is understandable, but over-simplification at the expense of accuracy is counterproductive, and does little to further learning.

JOHN A. J. GOWLETT
Research Laboratory for Archaeology
and the History of Art,
Oxford, UK

1. Halstead, L.B. *Nature* **288**, 208 (1980).
2. Wood, B.A. *Nature* **289**, 8 (1981).

NEWS AND VIEWS

Interferon: decline and stall

from Peter Newmark

THE good news to come out of a recent gathering in Rotterdam* was that, given to cancer patients, interferon does have some reasonably consistent clinical effects; the bad news was that there are the side effects of fever, malaise and weight loss. As the number of cases in trials of interferon increases from the tiny to the small and as the trials become randomized, controlled and double-blind, it is clear that interferon is no wonder drug. It remains, however, the case that the number of responses in most trials of interferon is above the threshold for declaring it useless. The consensus view is that interferon will eventually find a permanent place in the limited armoury of the cancer chemotherapist.

The evaluation of interferon in cancer, or for that matter as an antiviral agent, has been unconventional for two main reasons. The first is that it has been available in such small quantities; the second that it has such a marked species specificity that it has always been considered necessary to produce human interferon for human therapy and it has generally been thought pointless to test human interferon on experimental animals. Hence the first very impure products of the heroic Finnish effort, led by K. Cantell, to purify interferon from white blood cells were tested on minute numbers of patients and far too much was sometimes read into the occasional, dramatic response.

Although the supplies of interferon are increasing exponentially, many groups are still testing and reporting handfuls of cases without adequate controls. Among the best current trials of interferon are those of the American Cancer Society (ACS), all of which make use of the Finnish human leukocyte interferon (a mixture of α -interferons). In summarizing the ACS metastatic breast carcinoma trial, Dr E. Borden (University of Wisconsin) included the results of a parallel trial of the same interferon at the M. D. Anderson Hospital in Houston. Of the combined current total of 43 cases, 12 have shown a partial response — defined as one in which there is

at least a fifty per cent decrease in the sum of the diameters of all measurable tumours — and lesser responses were recorded in another 6 cases. Despite rumours to the contrary, Borden said the ACS trial will continue, although variations in the size, route and schedule of dosage may be introduced.

The ACS malignant melanoma trial appears less promising, with only one partial response and five lesser responses out of a current total of 43 cases (S.E. Krown). And in only 4 of 23 ACS cases of multiple myeloma was there registered a partial regression of disease (E.F. Osserman, Columbia University). Sadly this does not match conventional therapy and the ACS has decided that, henceforth, interferon will only be tried in combination with more active agents. For breast carcinoma there is still hope that interferon will turn out to be as good as any other single agent (but not as good as a combination of such agents) but it is too soon to be confident even of that conclusion.

Another relatively large trial of leukocyte interferon is on about 50 cases of osteosarcoma (H. Sander, Karolinska Hospital). This trial, unlike its promising predecessor, is being properly controlled and is not yet complete. Sander's impression was that interferon was proving to be better than conventional chemotherapy. Most other reported trials contained less than 10 patients except for a 26-case trial of fibroblast interferon (β -interferon) against neuroblastoma on which it is too early to pass judgement (D. Niethammer, University of Tübingen).

It is also too early to discover the results of the larger trials of interferon as an antiviral prophylactic agent. In particular, a proper trial of the ability of Finnish interferon to prevent herpes and papova virus infections of renal transplant patients (M.S. Hirsch, Boston) will take at least another year to complete. Meanwhile there is still guarded optimism for the use of interferon in the treatment of hepatitis B and both R. Sundmacher (University of Freiburg) and E. De Koning (Utrecht) reported that trifluorothymidine, in com-

bination with Finnish interferon, was markedly more effective against herpes virus-induced dendritic keratitis of the corneal epithelium than was trifluorothymidine alone.

As the clinical trials progress, so does the purity of the interferon tested. Purity can mean one of two things. The first is the extent to which contaminants have been removed; good preparations are now over eighty per cent pure and have a specific activity of over 10^7 international units per mg protein. But purity can also mean the separation of one subtype of interferon from a mixture. The only way to obtain a single member of the eight or so subtypes of α -interferon is from bacteria containing the relevant gene. Production by such means has advanced to the point where at least two small clinical trials of different subtypes have commenced with two hopes. One is that different subtypes will have distinct and complementary activities, as suggested by some *in vitro* tests (N. Stebbing, Genentech); the other is that some subtypes will be without side effects. In that respect it was the impression of T. Merigan (Stanford University) that subtype α_2 (from Genentech/Hoffmann La Roche) produced the usual side effects in man whereas H. Schellekens (TNO, Netherlands) claimed that the same subtype (from Biogen) did not produce the fever that leukocyte interferon did when given to the rhesus monkeys and yet was just as effective in the prophylaxis of vaccinia virus in that species.

Of great promise for the purification of individual interferons is the growing number of monoclonal antibodies which can be used in affinity chromatography. The original monoclonal (*Nature* **285**; 446, 1980) has now been joined by another one against α -interferon (L. Montagnier, Institut Pasteur) and several laboratories now have monoclonal antibodies against β -interferon (H. Erlich, Cetus Corporation; J. Trapman, Erasmus University; H. Hochkeppel, GBF Braunschweig).

Much attention is being turned to the most enigmatic of interferons, γ -inter-

Peter Newmark is Deputy Editor of *Nature*.

*'The Biology of the Interferon System', Rotterdam, April 21–24, 1981; organized by TNO in collaboration with the Erasmus University; proceedings to be published by Elsevier Biomedical around September 1, 1981.

feron. Previously known as Type II or immune interferon, this species is produced by cells of the immune system, particularly T cells. Production can be boosted by a variety of inducers and different laboratories have their favourite inducer. For example, R. Falcoff (Institut Curie) favoured anti-lymphocyte serum, H. Kirchner (*Nature* 291; 79, 1981) uses phytohaemagglutinin or concanavalin A on continuously cultured T cells, J. Vilcek's group swear by a combination of phytohaemagglutinin and phorbol esters (*Proc. natn. Acad. Sci. U.S.A.* 78; 1601, 1981), most recently mezerein (Y.K. Yip), which has less tumour promoter activity than the one previously used, and others favour protein A from staphylococci (for example, *Nature* 291; 77, 1981).

Induced cells are beginning to yield sufficient γ -interferon for its purification (or mRNA for its cloning). The big question is whether or not γ -interferon(s) will turn out to be distinct from other species in structure and activity. Conventional wisdom has been that γ -interferon is distinct but in a review of the literature L. Epstein (University of California, San Francisco) expressed caution in view of the impurities of all preparations so far tested. She finds no convincing evidence that γ -interferon has any unique effects on normal cell function and presented evidence that it induced the same 11 peptides in fibroblasts as did α -interferon. There remains, however, the possibility that γ -interferon is quite distinct; it has, for example, at least twice the molecular weight of other interferons (unless this represents polymerization) and is the only one to be unstable at pH 2. Unfortunately it also becomes very unstable on purification.

What, then, can be said of the future of the interferons as therapeutic agents? The consensus view appears to be that they will find a place in the treatment and prevention of viral diseases and in the treatment of cancer. For the latter they offer a unique therapeutic approach because they act not as toxins, directly on the tumour cells, but through the immune system, particularly natural killer cells. It is because of this that there is some optimism that γ -interferon, itself from cells of the immune system, will turn out to be a more potent anti-cancer agent than the other interferons. If the problem of side effects (see, for example, G. Scott *et al. Br. med. J.* 282; 1345, 1981) can be overcome, much larger doses than in the current typical schedule (10^6 units daily for 30 days and thereafter less frequently) may also improve the efficacy of interferons; already some patients have tolerated repeated doses of 100×10^6 units. Although interferon is not the wonder drug that many had hoped, though few had promised, a reasonable, positive view, as expressed by G. Galasso (NIH), is that we should at least be thankful for the arrival of a new drug which is active against diseases for which there are usually few, if any, therapeutic agents. □

Palaeoclimates north and south

from M. James Salinger

FOLLOWING the discovery that changes in Southern Hemisphere sea surface temperatures preceded changes in the Northern Hemisphere by about 3,000 years (J.D. Hays, X INQUA Congress, Birmingham, 1977), research into Southern Hemisphere palaeoclimates has accelerated rapidly. New data, presented at the first CLIMANZ* conference, allow a comparison between palaeoclimatic trends over a large area of the Southern Hemisphere with those of Europe and North America.

In the Northern Hemisphere the last glacial period commenced with a cooling at about 70,000 BP followed by a long period of lower temperatures. This period of cooler climates, continental ice masses and lower sea level was punctuated by a complex series of interstadials, with maximum ice volume at about 18,000 BP. Warming then occurred with very rapid temperature rises between about 15,000 and 12,000 BP. A more gradual rise occurred after 10,000 BP with the warmest period in the Holocene at about 6,000 BP.

Oxygen isotope measurements from the Huon Peninsula in northern New Guinea (P. Aharon, Australian National University (ANU)) show that relatively rapid fluctuations occurred in the average annual temperatures between 53,000 and 37,000 BP, with a maximum cooling of about -6°C below present temperatures during a marine regression at 43,000 BP. At 29,000 BP when sea level was relatively high (-45 metres) temperatures were $2.6 \pm 0.7^\circ\text{C}$ less than at present. These results can be compared with those from deep-sea cores analysed by CLIMAP which suggest a maximum cooling of about 2°C in the western Pacific. Lake data (J. Bowler, ANU) indicate a hydrological regime which

was radically wetter than present around 30,000 BP and earlier.

The New Zealand glacial record (T. Chinn, Ministry of Works, Christchurch) suggests that the maximum advance of the Pleistocene occurred around 23,000 BP and continued until after 21,000 BP, with a depression in annual temperatures, deduced from glacial deposits, of approximately 5°C . Corroborative data came from J. Hays (Lamont-Doherty Geological Observatory); faunal evidence from a deep-sea core at 42°S 80°W indicates a sea surface temperature of 8°C below present at 21,000 BP. Hays speculated that the greatest extent of Antarctic sea ice occurred around this time. These results seem to confirm that Southern Hemisphere changes lead those in the Northern Hemisphere by about 3,000 years, at least for the last glacial maxima.

A little later, around 18,000 BP, sand dunes were active in Australia, suggesting a period of increased windiness (J. Bowler, J. Ash and R. Wasson, ANU). Stronger westerlies displaced to the north are indicated by the distribution of quartz grains off the east coasts of Australia and New Zealand (see J. Thiede *Geology* 7; 259, 1979). These results, together with CLIMAP sea surface temperature data presented by Hays, imply that, at this time when the globe was extremely cold and Northern Hemisphere ice masses were at their maximum extent, the Southern Oscillation, an important feature of the atmospheric general circulation, was much reduced. This would have other implications, not least being a marked reduction in the frequency of upwelling along the South American coast (the El Niño phenomenon).

Comparison of climatic trends in Europe and North America with Australia and New Zealand

Time period (Before present)	Europe and North America	Australia and New Zealand region
40,000–25,000	Fluctuating but cool temperatures	Fluctuating but cool temperatures
23,000–20,000	Cooling	Maximum coldness
18,000–17,000	Maximum coldness	Cold
15,000–12,000	Rapid warming around 13,000–12,000	Rapid warming at about 14,000
10,000–8,000	Warming	Maximum warmth
Approximately 6,000	Maximum warmth	Slight cooling

*The CLIMANZ (Climatic Change of Australia and New Zealand) conferences aim to bring together groups of workers trying to reconstruct late Quaternary Australia and New Zealand climates.

M. James Salinger is in the Climatic Research Unit, University of East Anglia.

Between 15,000 and 10,000 BP a dramatic change in climate occurred. Geomorphic and palynological evidence from New Guinea indicates that deglaciation started before 14,500 BP with a rise in temperature amounting to 6°C over the entire period (G. Hope, ANU). Glacial changes in New Zealand were extremely rapid, retreating from near-maximum conditions around 17,500 BP to conditions similar to today by 13,500 BP (T. Chinn). At around 15,000 BP there was no substantial area of lowland forest in New Zealand but 5,000 years later both islands were almost completely forested (M. McGlone, Department of Scientific and Industrial Research, Christchurch). In the sub-Antarctic, Hays provided evidence of sea surface temperatures from a deep-sea core at 42°S 80°W. At around 15,000 BP temperatures were near to the glacial minimum and by 10,000 BP they had attained values near to Holocene maximum levels. His data from 42°S suggest that south of the oceanic Polar Front the sea ice regime changed extremely rapidly. Over 300 years at about 14,000 BP there was a sharp reduction in sea ice cover.

The maximum Holocene warming in the CLIMANZ area occurred at 9,000 BP, adding to the mounting evidence that the warmest, most pluvial periods in the past 15,000 years did not occur simultaneously throughout the globe. The warmest period in Europe is thought to be about 6,000 BP. Pollen from Mt Wilhelm and Mt Carstensz in New Guinea (G. Hope) provides evidence that tree-lines there were higher around 9,000 BP than at present. Data from much of southeastern Australia suggest warmer and wetter conditions.

Stratigraphical and pollen records from a number of sites in the southeastern highlands show highest temperatures and precipitation levels at 9,000 BP (A.P. Kershaw, W. Southern, J. Williams and L. Joyce, Monash University), coeval with high lake levels noted at Lake Frome (J. Bowler). At this locality the pollen analyses of G. Singh (ANU) indicate a summer monsoonal rainfall with annual rainfall of perhaps three times the present total. Between 10,000 and 8,000 BP New Zealand had a more complete forest cover than it had at any other time in the past 80,000 years (M. McGlone). The forests in the North Island grew under wet, mild conditions. Maximum forest cover in southern New Zealand, however, coincided with warmer and drier conditions than present. The deep-sea core at 42°S 80°W indicates a minimum of sea ice at this time. Again Southern Hemisphere temperatures in the CLIMANZ area at least seem to lead those in the Northern Hemisphere by about 3,000 years.

The evidence from moisture indicators show striking similarities with anomaly patterns in Australasia associated with an increased Southern Oscillation (Salinger *Mon. Weath. Rev.* **108**; 1892, 1980; Coughlan *Q. Jl R. met. Soc.* **105**; 707,

1979). The suggestion of a high Southern Oscillation Index around 9,000 BP would imply a stronger Walker Circulation and more frequent cold water upwelling off the South American coast and would have other consequences which are open to independent testing at other locations.

Parallels between the spatial patterns of past climates, and those of today, es-

pecially associated with important and well defined features of the general circulation such as the Southern Oscillation, provide a relatively simple way of interpreting local changes in a global context and may help us to understand more fully the lack of synchronicity between Northern and Southern Hemisphere climate changes over the past 50,000 years. □

Hooked on microtubules

from Jeremy Hyams

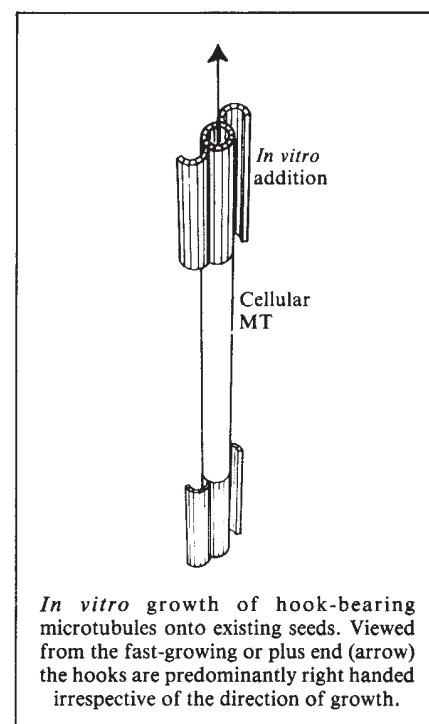
IN addition to their well documented role in ciliary and flagellar movement, microtubules also participate in many forms of intracellular motility. Phenomena such as the separation of chromosomes at mitosis, the redistribution of pigment granules in fish and amphibian chromatophores and transport along nerve axons are all spatially associated with arrays of microtubules and, in most cases, disrupted by anti-microtubule drugs such as colchicine.

A striking feature of these microtubule-associated movements is their bidirectionality; this property has been taken by many workers to reflect the organization of the underlying microtubules. Briefly, it has been suggested that there exist, in such situations, two families of microtubules with opposed polarities, each capable of exerting force in a given direction. Establishing the polarity of cytoplasmic microtubules has therefore long been regarded as the key to understanding the mechanisms of intracellular motility in much the same way as defining the orientation of the structural proteins of the muscle sarcomere was crucial to defining their interaction during muscular contraction.

Unfortunately for proponents of this view, until now there has existed no unambiguous morphological marker of microtubule polarity analogous to the arrowhead decoration of microfilaments by heavy meromyosin. Experimental dissection of microtubule directionality has therefore progressed only by the less expedient analysis of microtubule elongation *in vitro*. When tubulin subunits are added to a preformed seed (a fragment of microtubule or an organelle such as a flagellar axoneme or basal body), growth is favoured at one end (the fast or plus end) but also occurs to a lesser extent at the other (slow or minus) end (*Nature, News and Views* **284**; 402, 1980).

An important extension of this type of approach was introduced by Heidemann and McIntosh (*Nature* **286**; 517, 1980),

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who exploited an earlier finding that tubulin, polymerized *in vitro* in a high-ionic strength buffer containing dimethyl sulphoxide, formed not microtubules but sheets of protofilaments (Burton & Himes *J. Cell Biol.* **77**; 120, 1978; Mandelkow & Mandelkow *J. molec. Biol.* **129**; 135, 1979). When Heidemann and McIntosh used existing microtubules to seed this assembly, the polymer formed consisted of whole microtubules to which a C-shaped ribbon of protofilaments had attached, forming a conspicuous hooked appendage. Cross-sectioned images of microtubules grown on *Tetrahymena* basal bodies (see figure), viewed from their plus towards their minus ends, revealed that the hooks had a predominantly right-hand curvature, irrespective of whether microtubules were growing outwards or inwards from the basal body. The method therefore reveals not the direction of growth of these microtubules but, rather, their intrinsic polarity.

This exciting discovery was limited in its

applications to those microtubules which could be elongated *in vitro*. Now, however, by minor modification of their original method, McIntosh and his colleagues have shown that hooks can form directly on existing microtubules (Euteneuer and McIntosh *J. Cell Biol.* **87**; 509, 1980; *Proc. natn. Acad. Sci. U.S.A.* **78**; 372, 1981). In other words, the long-awaited morphological marker of microtubule polarity is at last available.

Application of the new technique to motility-related microtubules has already achieved some notable successes. Decoration of spindle microtubules from both plant and animal cells has sufficiently resolved earlier uncertainties concerning spindle organization to signal the demise of at least one hitherto highly regarded model of mitosis.

Results with other cellular systems have equally demanded that earlier views of microtubules in relation to intracellular motility be reconsidered. In the fish chromatophore, hook curvature was found to be the same for all microtubules and consistent with their plus ends being distal to the cell centre. Thus, even in situations in which transport is clearly bidirectional, the underlying microtubules all possess a single polarity. McIntosh and his colleagues conclude that microtubules do not contribute directly to motility in this system but, rather, form a vectorial framework upon which some other force-generating component is organized. Defining the interaction of microtubules with obvious candidates for this role, such as microfilaments and microtrabeculae, clearly now becomes a major challenge. □

of different kinds can be maintained separately and then combined catastrophically to provide explosive volcanism, such as was recently observed in Mount St Helens, and historically at Krakatoa (AD1883) and Santorini (BC1450).

Assuming reasonable physical parameters, the Rayleigh number at which convection would occur within a magma would be obtained in quite small chambers, about 50 m deep, although differences in, for example, viscosity and composition mean that convection could occur on the range of macro- and microscales commonly encountered in geological situations. As the magma convects to lose its heat, there is magma flow past the cooling front at which crystallization is being initiated. Solidification therefore takes place at this front as a function of its temperature and the compositions of the crystallized minerals and the liquid melt. Because the convection is unsteady, there are repeated changes in the composition of the minerals being deposited along the front giving rise to layering. Rice's model predicts that a basaltic magma chamber some 1 km deep would develop layers, each of which would be 5–10 m thick — as observed in the Skaergaard intrusion. One major difference to the previous models is that the layering would occur equally on the floor, ceiling and walls of the magma chamber, as is observed.

Rice's mechanism also explains the apparent close relationship between explosive silicic volcanicity and quieter basaltic eruptions. It has always seemed strange that basaltic lavas can be extruded through the flank fissures of a volcano that was, almost simultaneously, exhibiting violent silicic eruptions at its central cone. (The mechanism may also help to explain the puzzling 'Daly gap' in the range of compositions, between tholeiitic and rhyolitic, observed in many oceanic volcanic systems.) In the later stages of the proposed model, there is a high-viscosity, low-density, low-melting point rhyolitic magma overlying a low-viscosity, higher-density, higher-melting point basaltic magma. Rice shows that such a system is stable, apart from some convective intermixing in the boundary zone, until the top of the basaltic magma cools to its crystallization temperature. At this time, the basaltic magma will begin to exsolve volatiles that further reduce its viscosity and its density. This essentially destroys the stability of the system so that there is a near-instantaneous, massive, explosive 'roll-over' of the two magmas, often associated with the generation of superheated 'flashing', particularly if the magmas are hydrous.

Such a model, if valid, should allow better evaluation of the gestation time of the violent igneous eruptions and clarify how to estimate the potential hazard from the compositions of previously erupted phases. However, one major aspect in this proposed mechanism is that eddy-sized

Magmatic snap, crackle and pop

from D. H. Tarling

GRAVITATIONAL segregation of crystals out of a magmatic melt has long been considered to be the prime mechanism by which the composition of one or possibly two primary magmas can evolve to account for the observed varieties of igneous rock. This mechanism was originally proposed by Bowen (*Am. J. Sci.* **29**; 175, 1915) as a result of experiments in which olivine glass melts were held for 15 minutes at 1,430°C, before quench cooling, in a pot 2.5 cm high and 1.5 cm wide. The fact that gravitational segregation of individual crystals in a magmatic melt is physically impossible has, however, only been appreciated by a few researchers and the mechanism has only received wide acceptance because there appeared to be no satisfactory alternative.

The actual mechanism by which the fractionation takes place is absolutely vital to any interpretation of the isotopic ratios in igneous rocks. In particular, it is widely accepted that $^{86}\text{Sr}/^{87}\text{Sr}$ ratios are low for rocks of mantle derivation. Such a view is based on the assumption that normal oceanic ridge basalts are representative samples of the mantle — that the isotopes themselves have not been partitioned to any significant extent during the formation of the oceanic crust. A recent paper on convective fractionation by Rice (*J. geophys. Res.* **86**; 405, 1981) is therefore critical not only to the understanding of igneous petrological evolution models, but also to the whole basis of geochemical analyses of igneous rock types. It also provides a satisfactory explanation for the occurrence of violent, explosive volcanism associated with the sudden mixing of radically different magma types.

The fundamental objection to crystal segregation is that crystals forming within a cooling magma would take an infinite time to reach the bottom of the magma chamber and so could not accumulate as a crystal mush at the bottom. In any case, the evidence from layered igneous complexes, such as the Skaergaard intrusion of Eastern Greenland, is that the layering takes place on the roof and sides of the chamber as well as on its floor, and it can be repetitive throughout the sequence. To account for such observations, both Hess (*Geol. Soc. Am. Mem.* **80**; 230, 1960) and Wager and Brown (*Layered Igneous Rocks*, Oliver and Boyd, Edinburgh, 1968) proposed that the layering was associated with turbidity currents within the magma chamber, from which individual crystals then segregated. However, although some sedimentary features can be found in some layered complexes, many such features are absent — apart from the intrinsic impossibility of sinking individual crystals through such a viscous medium and the necessity to plate both the walls and ceiling, as well as the floor.

What, then, does Rice propose to replace this widely accepted but impossible model? Essentially he draws on his industrial background in glass and metallurgical processing, combines this with studies by Hurler (*J. Crystal Growth* **13/14**; 39, 1972), and proposes a convective process of fractionation that also explains layering on a range of scales, and shows how magmas

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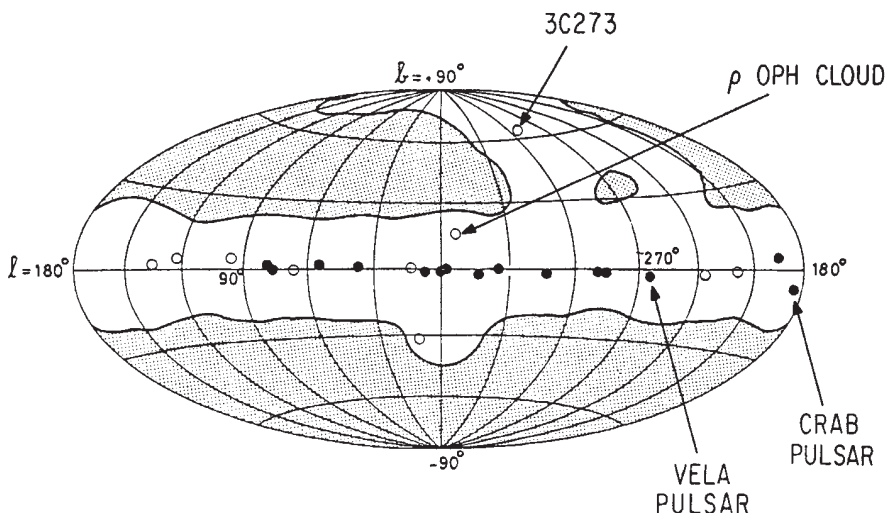
A catalogue of unidentified objects

from David J. Thompson

THE sky seen with a gamma-ray telescope offers a unique view of high-energy astrophysical processes. This uniqueness is emphasized by the second COS B catalogue of high-energy gamma-ray sources (Swanenburg *et al. Astrophys. J. Lett.* 243; L69, 1981) which documents the remarkable lack of obvious correlation between most of the gamma-ray sources and previously known astronomical objects.

The high-energy gamma-ray telescope aboard ESA's COS B satellite has been in operation for nearly six years and data used in compiling the second COS B catalogue span more than three years. The 25 sources of gamma rays with energies above 100 MeV which make up the catalogue may be either stellar or extended sources (with diameters up to 2°). Although the COS B survey does not cover the entire sky, it does include the full galactic plane, along which most of the sources are clustered. There is also a concentration towards the inner part of the Galaxy. In addition to the simple observation that these sources are galactic, their spatial distribution suggests that they lie within 2 to 7 kiloparsecs of the Solar System and, hence, are radiating on the order of 10^{36} erg s^{-1} in the form of high-energy gamma rays.

Despite efforts by a number of investigators, only four of the 25 sources have firm identifications. The Crab and Vela pulsars are recognizable by their time signatures, having the same periods as the radio pulsars. The quasar 3C273 and the interstellar cloud complex near the star ρ Ophiuchus are identified by positional coincidence, which is meaningful only for these high-latitude sources. An extended source, not appearing in the catalogue, has also been



The second COS B catalogue of high-energy gamma-ray sources, shown in galactic coordinates. Observations covered the unshaded region. Filled circles denote sources with measured fluxes $\geq 1.3 \times 10^{-6}$ photons (>100 MeV) $cm^{-2} s^{-1}$, while open circles denote sources below this threshold (from Swanenburg *et al.* 1981).

identified by COS B with the molecular clouds in Orion (Caraveo *et al. Astr. Astrophys.* 91; L3, 1980). The remaining 21 members of the catalogue are distinguished by their lack of counterparts at other wavelengths. In particular, the gamma-ray luminosities of these sources are at least an order of magnitude greater than their X-ray luminosities and several orders of magnitude greater than their radio luminosities. This preferential production of gamma rays indicates an unusual astrophysical setting, dominated by large energy transfers. The fact that the identified gamma-ray sources are not all of the same type suggests that the unidentified sources may themselves not be a homogeneous group. The variety of energy spectra seen for the sources in the COS B catalogue is a further indication that two or more classes of gamma-ray source may emerge from future studies.

The intriguing possibility is that at least some of these presently unidentified sources may turn out to be a totally new class of galactic object.

Further results can be expected from COS B, particularly in the area of time variability studies, but positive identification of these mysterious sources depends on future observations. The Soviet-French GAMMA-1 satellite (to be launched in the next two years) should give improved position information for some of these. The instruments selected for the Gamma Ray Observatory (now scheduled for launch in the latter part of the 1980s) have the broad energy range, good angular resolution and high sensitivity to provide the next breakthrough in gamma-ray source studies. The importance of the COS B catalogue is that it establishes a sizeable number of gamma-ray sources with astrophysically unique properties.

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convective systems, particularly those associated with magmatic fluids within cracks, are likely to be characterized by a Soret type of isotopic fractionation (as used to produce enriched uranium) that could drastically modify the ratios of even relatively stable, immobile isotopes. The lack of evidence for significant magma chambers beneath the Atlantic and Indian Ocean ridges certainly indicates that the fractionation of the mantle melt to form the oceanic crust probably operated predominantly within tensional cracks in the lithosphere, rather than in a clearly defined magma chamber. The magma chambers

thought to have been identified along the East Pacific Rise could, in fact, be areas of the mantle with intensive fractures filled with magmatic fluid, rather than the conventional concept of a large volume of magmatic liquid. Even if magma chambers do exist in such situations, Rice's model could mean that there is much more isotopic fractionation of mantle materials than has generally been recognized. Thus mantle homogeneities postulated on the basis of variation in elements and their isotopes from mid-oceanic ridges to off-centred volcanoes could merely indicate slight differences in the Soret fractionation

system in the different areas. Much of the evidence for or against mantle sources for many magmas could similarly be at least gross oversimplifications.

The implications of Rice's model are far-reaching. His paper highlights our ignorance of the fractionation processes in magma chambers, and demonstrates the inadequacy of widely accepted, but incorrect mechanisms for fractionation. It could also mean that we are only just beginning to understand the ways in which igneous rocks are formed and that all previous geochemical interpretations must be re-evaluated. □

What are mast cells for?

from T. M. Dexter, R. W. Stoddart and S. T. A. Quazzaz

FROM the time that Paul Ehrlich first described the presence of water-soluble, metachromatically staining granules in certain cells after treatment with Toluidine blue¹ to the present day when Astra blue is used to distinguish the same granules², staining properties have been the basis for distinguishing tissue mast cells and basophils from other cell types. Indeed for many years these cells were known only as morphological entities whose numbers were increased in certain pathological conditions. Mast cells are present in all mammals so far studied and have a diffuse distribution, being detected in the gut mucosa, peritoneal cavity, subcutaneous tissue lymph nodes and the thymic capsule. Although the precise relationship between circulating basophils and tissue mast cells is unclear, one may be derived from the other.

The origin of these cells has long been a matter of speculation. Burnett suggested³ that at least some mast cells represent post-mitotic derivatives of T lymphocytes, citing evidence such as the response of mast

cells to T-cell mitogens, mast cell reactions during the response to parasitic infections, and the ease with which mast cells can be generated from cultures of lymphoid tissue. As early as 1963, Ginsburg and Sachs⁴ showed that mast cells were regularly produced when thymus cells were cultured on fibroblast feeder layers and others^{5,6} suggested that mast cells in the gut mucosa are derived from T cells. However, these suggestions are not compatible with the finding that normal numbers of mast cells are present in the subcutaneous tissue of athymic (*nu/nu*) mice which are devoid of T cells⁷.

Kitamura and co-workers⁸ have used the elegant system provided by the *bg⁺/bg⁻* mice, where the peripheral blood leukocytes have highly characteristic giant granules (Chediak-Higashi syndrome). Using the granules as cell markers in marrow transplant chimaeras, they showed that mast cells were derived from marrow precursors, and that mice with a genetically determined macrocytic anaemia (*W/W^v* mice), associated with a defect in the pluripotential haematopoietic stem cell, also lack tissue mast cells⁹. *W/W^v* mice are useful in that they will accept marrow grafts—which will cure the anaemia—without the need for prior irradiation to ablate the endogenous haematopoietic

system. The availability of these two mouse strains with genetic markers has now paved the way for a direct analysis of mast cell development.

In the experiments reported in this issue of *Nature* (p.159), Kitamura *et al.* have exploited the ability of murine haematopoietic stem cells to undergo clonal proliferation and differentiation and form colonies in the spleens of potentially lethally irradiated mice. A mixture of *bg⁺/bg⁻* and wild-type stem cells was injected into wild-type mice. The origin of the resulting spleen colonies was determined using the *bg⁺/bg⁻* giant granules as a cell marker, and cells from isolated, individual spleen colonies (containing a 'clonally' pure cell population of either *bg⁺/bg⁻* or wild-type cells) were injected into *W/W^v* mice. Several months later, the blood haemoglobin type was used to determine whether the *W/W^v* recipients had been reconstituted with cells derived from individual spleen colonies. The animals were then killed and the tissue mast cells and granulocytes examined. In many animals, mast cells which were wholly *bg⁺/bg⁻* or wild type appeared in the tissue — and in one mouse the mast cells, erythroid cells and some granulocytes were shown to be *bg⁺/bg⁻* derived. These data show fairly conclusively that mast cells and myeloid cells have a common origin in the pluripotential haematopoietic stem cells, but note that the long time interval (15 weeks) between reconstitution of the *W/W^v* mice and analysis of the mast cells leaves open the possibility that thymus processing is an obligatory step for mast cell development.

This possibility has now been excluded, however, by the recent work of Schrader *et al.*¹⁰. These authors describe a persisting (P) cell (a name which one can only hope will be replaced before it becomes established!) generated in agar cultures of myeloid progenitor cells and which can be grown in liquid cultures for long periods, provided that the cells are supplied with an appropriate stimulating factor (in this case a conditioned medium derived from concanavalin A-stimulated T cells). In the absence of this factor, the cells die. Such cells contain granules which stain with Astra blue, possess histamine and appear to be equivalent to mast cells. They lack lymphoid markers and can be grown from marrow culture of *nu/nu* mice, from

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100 years ago

We take the following from the *Colonies and India*: "To say that a train had been stopped by caterpillars would sound like a Yankee yarn, yet such a thing (according to the *Rangitikei Advocate*) actually took place on the local railway a few days ago. In the neighbourhood of Turakina, New Zealand, an army of caterpillars, hundreds of thousands strong, was marching across the line, bound for a new field of oats, when the train came along. Thousands of creeping vermin were crushed by the wheels of the engine, and suddenly the train came to a dead stop. On examination it was found that the wheels of the engine had become so greasy that they kept on revolving without advancing — they could not grip the rails. The guard and the engine-driver procured sand and strewed it on the rails, and the train made a fresh start, but it was found that during the stoppage caterpillars in thousands had crawled all over the engine, and over all the carriages inside and out."

Messrs. Siemens and Halske gave a public trial last week of their new electric railway, which runs between Lichterfelde and the Cadettenhaus, about six miles from Berlin. The trial is

stated to have been most successful. It was in a simple tramcar, with an electric battery totally concealed between the wheels, in connection, through the rails it ran on, with the principal battery at the station. The rails are three feet three inches apart, and exactly resemble those of an ordinary railroad, only the gauge behind narrower. The greatest speed obtained on a distance of about one and a half miles was eighteen English miles an hour. Dr Siemens has proved that if necessary a far greater speed could be obtained, but this is not allowed by the German police authorities. It will not be allowed to proceed at more than nine miles per hour. The railway was opened to the public on Monday.

In connection with the subject of "Fascination," Dr. Otto of Schemnitz, Hungary, writes us of a case which came under his notice. In 1859, when the use of firearms was under stringent regulations in Hungary, peasants often killed hares on the Danubian island Creppel in the following way:— Two peasants would drive in a cart over the reaped fields. On spying a hare (say at two to three hundred paces) they proceeded to drive round it some five or six times in succession. One peasant carrying a long stick at length sprang out, at the moment the cart was behind the hare (the cart continuing its course), and coming up to the animal slowly, killed it without difficulty. It was not uncommon to kill thus as many as six or seven hares in one morning.

from *Nature* 24, 12 and 19 May, 1881

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precursor cells which can also produce granulocytes. These cultured mast cells should allow analysis of the variety of responses in which mast cells are thought to be involved. However, tissue mast cells appear to be functionally heterogeneous and the 'P' cells may represent a developmentally restricted sub-population.

What is the function of mast cells? For many years they have been implicated in hypersensitivity reactions¹¹ and many parasites, such as schistosomes, are known to stimulate mast cell accumulation. After such infections there is a rapid and dramatic increase in the number of mast cells in the lymph nodes (mainly in the cortical and capsular regions) followed eventually by a decline. The initial accumulation is thought to represent migration of mast cells to the lymph nodes; and the 'disappearance' is believed to be due to degranulation rather than exit via the lymph node hilus¹². Whether the degranulated cells leave the node in some unrecognized form and recirculate is not known. Normally, mast cells are rather more abundant in the medulla and the deeper paracortex than in the peripheral

parts of the node; they are strikingly absent from germinal centres. In some pathological conditions a distribution similar to that seen in parasitic infections is found, again suggesting centripetal migration. What the mast cells are doing in the lymphoid organs is open to speculation.

Most cells contain significant amounts of histamine and many cells, including lymphocytes, have histamine receptors¹³. Histamine inhibits lymphocyte-mediated cytotoxicity by T cells¹⁴, possibly indicating a role for mast cells in modulating T-cell function. In this context it is significant that T cell-deficient *nu/nu* mice, which have normal or increased numbers of mast cells in subcutaneous tissue, nonetheless show a complete failure of intestinal mast cell response to parasitic infections¹⁵. However, adequate function can be restored with a thymus graft, perhaps indicating that a T-cell helper function is required. Obviously Schrader's 'P' cells may help to unravel this sort of cooperative response.

Mast cells contain within their granules amines, such as serotonin, and can release

large amounts of prostaglandins and leukotrienes. They also specifically produce heparin, a highly sulphated glycosaminoglycan. Heparin has been implicated in the sequestration of lipids by tissue cells and stimulates the migration of capillary endothelial cells¹⁶. The tissue distribution of mast cells suggests that they may play an important part in the production of heparin and local angiogenesis. Since the successful growth of a tumour depends on its blood supply, this is likely to be of profound importance in the determination of patterns of tumour growth. For example, the growth of spherical tumour masses with necrotic centres might be related to a high density of mast cells immediately around, but not within, the tumour mass.

It is apparent that the careful investigation of the occurrence of mast cells in a variety of pathological conditions is likely to reveal new facets in their role in chronic inflammation, response to injury and the initiation of repair. It is already clear that they are much more than pharmacological time bombs or land-mines simply to be tripped by wandering molecules like IgE. □

Leishmaniasis and mouse genetics

from F. E. G. Cox

THE paradox that in some forms of leishmaniasis there is a massive antibody response but not protection, while in others there is no antibody response but excellent protection, is gradually being explained by the use of genetically defined strains of mice.

Leishmaniasis is one of the six diseases selected by the WHO for its Special Programme for Research and Training in Tropical Diseases and is the general name given to infection with any one of some five species or 14 subspecies of the protozoan genus *Leishmania*. Leishmaniasis is transmitted by sandflies and the parasites injected into the human host multiply by repeated binary fission within macrophages in various parts of the body. The manifestations of the disease range from simple, slowly healing cutaneous lesions to fatal disseminated infections involving the viscera. In the Old World the cutaneous form is caused by *Leishmania tropica* and the disseminated visceral form by *Leishmania donovani*. In the New World *L. tropica* is replaced by a number of species that affect not only the skin but also underlying tissues.

At present there are no fully effective drugs or methods of vector control for the prevention of leishmaniasis and no vaccine at all. Fortunately, the parasites can infect mice, so recent studies using these animals have provided a wealth of information that

may eventually be useful in the control of leishmaniasis.

Resistance to *L. donovani* and *L. tropica* is genetically controlled — some strains of mice are very resistant to infection while others are susceptible¹⁻³. In *L. donovani* infections resistance is controlled by a single autosomal gene on chromosome 1, designated *Lsh*. Differences between resistant (dominant) and susceptible (recessive) mice appear before day 50⁴. Among the susceptible mice some exhibit prolonged infections while others experience a dramatic fall in parasite load. This response is controlled by a gene or genes within or close to the H-2 region of chromosome 17 which allows some innately susceptible mice to recover⁵.

L. tropica infections, similarly, are under genetic control with some mice, such as the C57BL, being resistant and others, in particular BALB/c mice which experience fatal disseminated infections, being susceptible⁶. Resistance to *L. tropica* is controlled by a single autosomal gene which is neither the *Lsh* gene involved in *L. donovani* infections nor the H-2 complex, although H-2 genes may be involved late in the infection⁶. Immunity to *L. tropica* involves an antibody-mediated immune response of the delayed hypersensitivity type (DTH) in which the main cells concerned are T lymphocytes (T_D). In susceptible BALB/c mice the lack of immunity is due not to a failure to elicit a DTH reaction but to the induction of

suppressor T lymphocytes (T_S), cells which impair the DTH reaction⁷. Early in the infection, DTH is detectable but later it is suppressed. The pattern of events has been elegantly investigated by Howard, Hale and Liew⁸. They subjected BALB/c mice to sublethal irradiation at levels known to delete the precursors of T_S cells and found that the infection, instead of progressing to a fatal conclusion, began to resolve at the same time as those in genetically resistant mice.

Immunity to *L. tropica*, therefore, depends on a DTH reaction which in certain mice can be suppressed by T_S cells. What role, if any, does the humoral immune response play? In the experiments using irradiated mice⁸, irradiation at levels that ablate antibody production but not T_S cells had little effect on *L. tropica* infections. Antibody levels in both

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irradiated-healing and non-healing mice were similar. A more direct approach to the role of antibody has involved the use of Biozzi high- and low-responder mice. In Biozzi high responders (Ab/H) antibody levels are high and macrophage activity low while in low responders (Ab/L) the reverse is the case⁹. In Ab/H mice infected with *L. tropica*, large, progressive, ulcerating lesions are produced in the presence of high specific antibody titres while in Ab/L mice

the lesions are small and healing and antibody levels are minimal¹⁰. These results confirm unequivocally the widely held belief that in *L. tropica* infections the main protective mechanism is a DTH response and that antibody plays little or no part in recovery. The actual outcome of the infection depends on the interplay of T_D and T_S cells and in due course this information should give us some insight into why some human patients easily

overcome the infection and experience only an insignificant lesion while others suffer from spreading and sometimes fatal infections. These findings are relevant not only to *L. tropica* but also to other species. There are indications that similar mechanisms may be involved in infections with the New World species, *Leishmania mexicana*¹¹, and it may be that the failure to overcome *L. donovani* infections also involves a defect in the DTH mechanism. □

Interstellar grains: models and measurements

from David A. Williams

INTERSTELLAR GRAINS are small solid particles mixed with the interstellar gas which reveal their presence to astronomers in various ways: by causing extinction and polarisation of starlight, or by emitting in the infrared (IR). Extinction is caused by absorption, which heats the grains, or by scattering, which gives rise to an observable, diffuse radiation. Grains also have chemical effects on the interstellar gas, being responsible for conversion of atomic to molecular hydrogen by surface catalysis, and for heavy element depletion. Although grains have been discussed for more than forty years, a recent conference* showed that the questions astrophysicists ask about them have not greatly changed: what are they made of, how are they formed and destroyed, and what is their interaction with gas and radiation?

Astrophysicists have traditionally relied on the curve showing variation of interstellar extinction with wavelength as a pointer to grain identification. In reviewing visual and UV observations Wolstencroft (Royal Observatory, Edinburgh) emphasised the remarkable constancy of the extinction curve in different regions of the Galaxy, especially in visual wavelengths. Some variation does occur in the UV suggesting that (at least) the grain size distribution may vary. Whittet (Preston Polytechnic) showed from a study of the ρ Oph cloud that, contrary to previous reports, such variations are not accompanied by significant changes in the gas to dust ratio. Measurements of the diffuse (scattered) light in the visual and UV place conflicting constraints on grain properties. Duley (York University, Toronto) and Williams (UMIST) offered a solution in terms of an additional UV component arising from H₂ fluorescence that is not normally allowed for.

Participants were excited to hear from Scarrott (University of Durham) of several peculiar emission features longwards of 5,700 Å from the mysterious Red Rectangle. The origin of these features

caused much speculation and Greenberg (Rijksuniversiteit te Leiden) suggested an answer based on the laboratory simulation of grain mantles. Ices of simple gases (such as water, methane and ammonia) in approximately cosmic proportions are irradiated by UV and dramatic changes in the material are observed. Flashes of light may be emitted as warm-up occurs and the residue has optical features which Greenberg proposes are the diffuse bands.

Unidentified spectral features associated with grains also abound in the IR. Whittet reviewed recent observations of emission features from HII regions, planetary nebulae, galactic nuclei, and the absorption features in the cool interstellar gas. Most participants were convinced that the prominent 9.7 μ m feature (seen in emission at high temperature and in absorption) is to be attributed to the SiO bond in refractory material such as amorphous silicates or oxides. The 3.1 μ m feature, seen only in absorption in dark clouds, was generally attributed to molecular mantles accreted by cold grains.

A number of other IR absorption and emission features in the range 3–12 μ m are known and two alternative interpretations were given. Allamandola (Rijksuniversiteit te Leiden) presented results of laboratory measurements of absorption features from ices such as H₂O, CO and CH₃OH, and identified them with observed absorption features at 3.4, 3.5, 6.0 and 6.8 μ m. Duley and Williams suggested that radicals bound at reactive sites on amorphous carbon grains produce these and other absorption features, and also emission features from warm grains. The precise spectral properties of such grains will change according to their environment. Clearly, the flood of IR observations is providing a set of tight constraints on grain composition.

These and other constraints were discussed by Greenberg, who suggested that all observations so far were consistent with models of amorphous silicates or oxides, carbons, dirty ice mantles and photoprocessed material. The interaction of gas and grains can be studied in detail when the grain material is specified, for

then the vast surface chemical literature can be tapped. Duley showed how effectively this can be done. He described a consistent model, including depletion, chemical reactions and optical properties, with the attractive feature that the chemical and optical nature of grains change as grains move from one environment to another. Apparent changes in grain properties may merely reflect their physical state.

The formation of grains is indicated astronomically in a number of sources: late-type giants and supergiants, other special stars, planetary nebulae, novae and supernovae. Some problems remain in understanding nucleation processes. Mechanisms of destruction (sputtering, evaporation and shattering in collision) seem able to account for the life times of grains. Evans (University of Keele) reviewed observations of a nova exhibiting grain formation. There are some difficulties in explaining why the grain temperatures increased after the nova had already started to decline in brightness. There are also some quite puzzling observations of ejection in WR stars in which optical and IR intensities decline with time but the measurement at 3.4 μ m remains high.

The Durham group, in work described by Smith, demonstrated that sophisticated modelling of optical polarisation in reflection nebulae is particularly rewarding in understanding the nebulae and the grains. Robinson (Queen Mary College, London) convinced the workshop that in such modelling, especially of IR sources, workers must account correctly for radiative transfer or quite spurious interpretation concerning grains and source properties may result.

The meeting ended in a mood of some optimism. Although much controversy remained it was felt that new observations, particularly in the IR and UV, allied to sophisticated modelling, will enable significant progress to be made in the next few years. □

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*A workshop on interstellar grains was held in Manchester on April 7–8, 1981.

ARTICLES

Episodic granite plutonism in the Scottish Caledonides

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Boulders from a Lower Devonian conglomerate complete the record of a phase of Ordovician plutonism associated with island arcs and blue schist metamorphism, indicating high angle subduction. Following a phase of sedimentary accretion, late Silurian plutonism was the result of low angle subduction. The two granite suites have distinct isotopic trends.

BOULDERS of two-mica granite which occur as clasts in the Lower Old Red Sandstone conglomerates along the Highland Boundary Fault (Fig. 1) have yielded Rb–Sr muscovite ages of ~460 Myr confirming the existence of a widespread suite of subduction-related Ordovician granites south of the Great Glen Fault. A voluminous suite of late Silurian to early Devonian granites can be related to the subduction of comparatively younger, thinner, hotter and more buoyant oceanic crust. The intervening hiatus in plutonism coincides with the peak of subduction accretion in the Southern Uplands, further demonstrating the necessity of subducting wet sediments to generate granitic magmas. For the Ordovician suite, the sedimentary record completes an isotopic trend of increasing $^{87}\text{Sr}/^{86}\text{Sr}$ ratios away from the trench which can be related to the increasing

involvement of upper crust. The later granites show a trend towards more unradiogenic lead away from the trench which can be attributed to the increasing involvement of lower crust.

The Lower Old Red Sandstone rocks of the Midland Valley of Scotland comprise a thick sequence of late orogenic alluvium and intercalated volcanic rocks¹. The sequence at Stonehaven (Fig. 1a), possibly exceeding 8 km in thickness, contains abundant conglomerates which are dominated by igneous clasts² (Fig. 1b). Andesitic and basaltic clasts are particularly abundant and often less well rounded than accompanying granite clasts of similar size. Granite which locally makes up ~25% of the clasts, ranges from microgranite to pegmatites and is invariably primary muscovite bearing. These clasts have generally been considered to have been derived from the late Silurian–early

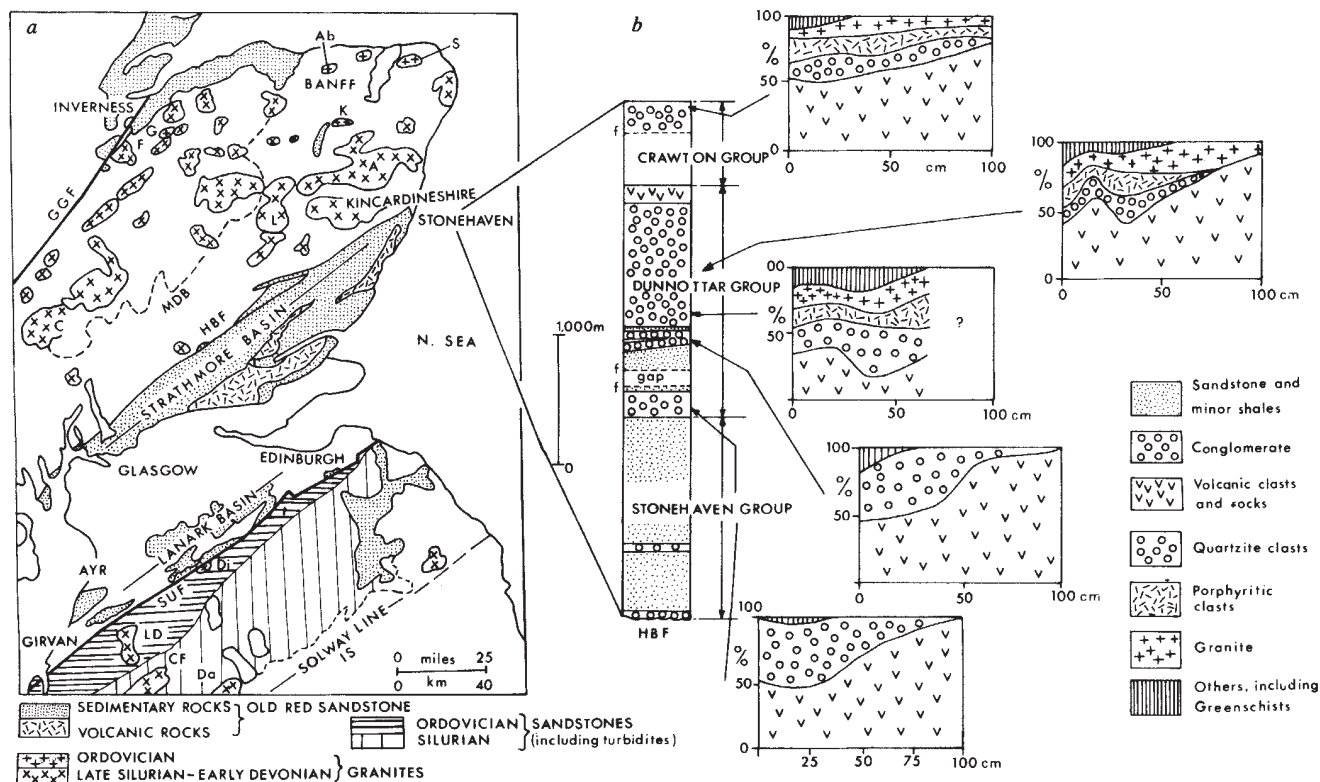


Fig. 1 a, Showing distribution of Caledonian granites in Scotland, the major tectonic lines and localities referred to in the text. Ordovician granites: Ab, Aberchirder; K, Kennethmont; S, Strichen; G, Glen Kyllachy. Silurian–Devonian granites: Da, Dalbeattie; CF, Cairnmore of Fleet; LD, Loch Doon; Di, Dinstinkhorn; A, Aberdeen Hill of Fare; L, Lochnagar; C, Cruchan; F, Foyers. Tectonic boundaries: IS, Iapetus Suture; SUF, Southern Uplands Fault; HBF Highland Boundary Fault; MDB, Moine Dalradian Boundary; GGF, Great Glen Fault. b, Stratigraphic sequence at Stonehaven–Crawton² and distribution of rock types-by-size through the sequence.

Table 1 Rb–Sr isotopic data

Sample	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}$ (atomic)	$^{87}\text{Sr}/^{86}\text{Sr}_i$ (atomic)	Mineral age (Myr) $\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$	$(^{87}\text{Sr}/^{86}\text{Sr})_i$ (atomic)
R.C. 1913						
Whole-rock	133	48.4	7.999	0.77254 ± 4		0.7196 ± 6
Muscovite	529	4.23	474.1	3.8585	465 ± 5	
Biotite	867	19.7	138.2	1.5536	421 ± 5	
R.C. 1916						
Whole-rock	172	116	4.287	0.74295 ± 6		0.7150 ± 3
Muscovite	521	22.5	70.15	1.1721	459 ± 5	
R.C. 1917						
Whole-rock	110	217	1.466	0.72127 ± 7		0.7116 ± 2
Muscovite	344	27.1	37.61	0.95881	461 ± 5	
Biotite	481	37.0	38.57	0.96406	459 ± 5	
Biotite	491	35.0	41.08	0.97964	458 ± 5	
R.C. 1919						
Whole-rock	142	206	1.999	0.72326 ± 4		0.7103 ± 2
Muscovite	339	20.8	48.68	1.0270	457 ± 5	
Biotite	649	17.8	112.7	1.4236	444 ± 5	
R.C. 1920						
Whole-rock	113	83.1	3.931	0.73790 ± 6		0.7122 ± 3
Muscovite	382	9.74	122.4	1.5127	459 ± 5	

Devonian granites to the north^{3,4}. However, the muscovite-bearing granite clasts become less abundant upwards in the succession.

Isotope analyses

Rb–Sr isotope dilution analyses (Table 1) follow analytical methods described elsewhere⁵. Rb–Sr muscovite ages for the four muscovite biotite granites RC 1916, 17, 19, 20 are concordant at 459 ± 5 Myr. The Rb–Sr muscovite age for pegmatitic sample RC 1913 may be slightly older at 465 ± 5 Myr. Rb–Sr biotite ages scatter down to 421 ± 5 Myr. The biotite age for sample RC 1917 is, however, concordant with the muscovite ages.

The Rb–Sr muscovite ages are here interpreted in terms of the time of emplacement of these underformed granites and pegmatite for two reasons. First, in the central Highlands and within the axial region of the Caledonian orogen, Rb–Sr muscovite systems have survived all phases of Caledonian deformation and associated amphibolite facies metamorphism⁶. Second, the

concordance of the biotite and muscovite ages for sample RC 1917 indicates that regional temperatures in at least some of the host rocks could not have greatly exceeded 300°C , the estimated blocking temperature for the Rb–Sr isotopic system in biotite⁷. The younger, scattered biotite ages are believed to have been caused by alteration to chlorite during deposition in lower Devonian time.

The wide range in initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios obtained is interpreted in terms of increasing upper crustal involvement. The chemistry of the rocks bears little systematic relation to the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios which suggests that the magmas sampled by the boulders did not involve simple mixing between two end members. Concentrations of SiO_2 range from 71 to 74 wt%, Na_2O from 3.1 to 3.9 wt%, K_2O from 3.5 to 4.8 wt% and normative corundum from 0.1 to 2.6 wt%. The wide range in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios combined with the lack of evidence for a mixing pattern suggests that the boulders came from more than one intrusion. Thus the tight grouping of Rb–Sr muscovite ages at ~ 460 Myr supports the evidence from the Stonehaven clast profile (Fig. 1) that these late Ordovician granites represent a distinct pulse of magmatism.

Granite clasts

The provenance of the ~ 460 Myr granite clasts is not known. The conglomerates in which they occur thicken onto the north-west limb of the Strathmore Syncline suggesting a source to the north-west, a view supported by the presence of Highland-type rocks in the conglomerates. In terms of durability during transportation, schists and volcanic rocks would probably break down quite quickly, granite less quickly and quartzite least of all⁸. On this basis it is estimated that granite formed some 10–15% of the exposed ground in the source at about the time the Dunnottar Group (near Dunnottar Castle) was laid down. The present areas to the north of Strathmore (in southern Aberdeenshire) contain some 43% granite bedrock by area (taking a strip of country 20 km to north-west of the Highland Boundary Fault for 60 km south-west from Stonehaven). These Silurian–Devonian granites have not been found in the Devonian conglomerates. The nearest rocks of ~ 460 Myr age are found some 60 km to the north in Banff⁹. In view of the size of the granite boulders (up to 80 cm) transport over this distance is unlikely, and a more proximal source either in the Midland Valley or in the southern Highlands is envisaged. Given no substantial lateral movement on the Highland Boundary Fault and accepting the stratigraphical evidence, we suggest that these early plutons were emplaced high into the Dalradian cover to the north, among rocks which were largely in greenschist facies metamorphism. These greenschist rocks provided some of the

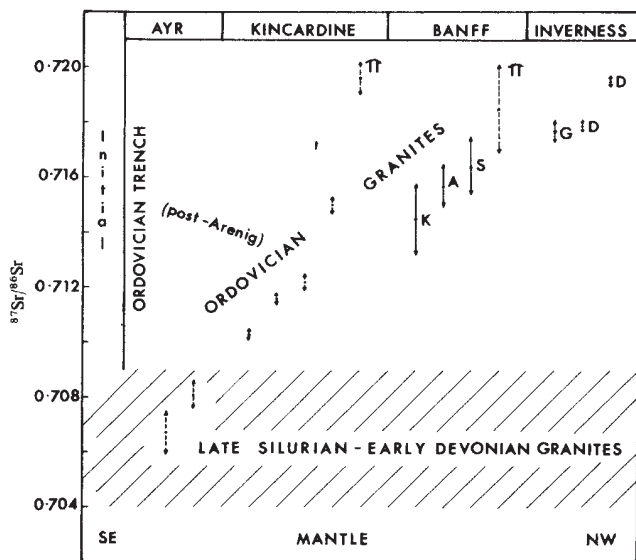


Fig. 2 Cumulative frequency plots of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for post-Arenig Ordovician granites in Scotland. From left to right regions have been arranged from the Ordovician trench into the interior of the continent. Dashed lines indicate uncertainties on individual whole rock: clasts, granodiorite dykes (D) or pegmatites (π). Solid lines indicate uncertainties on whole rock isochrons. See Fig. 1.

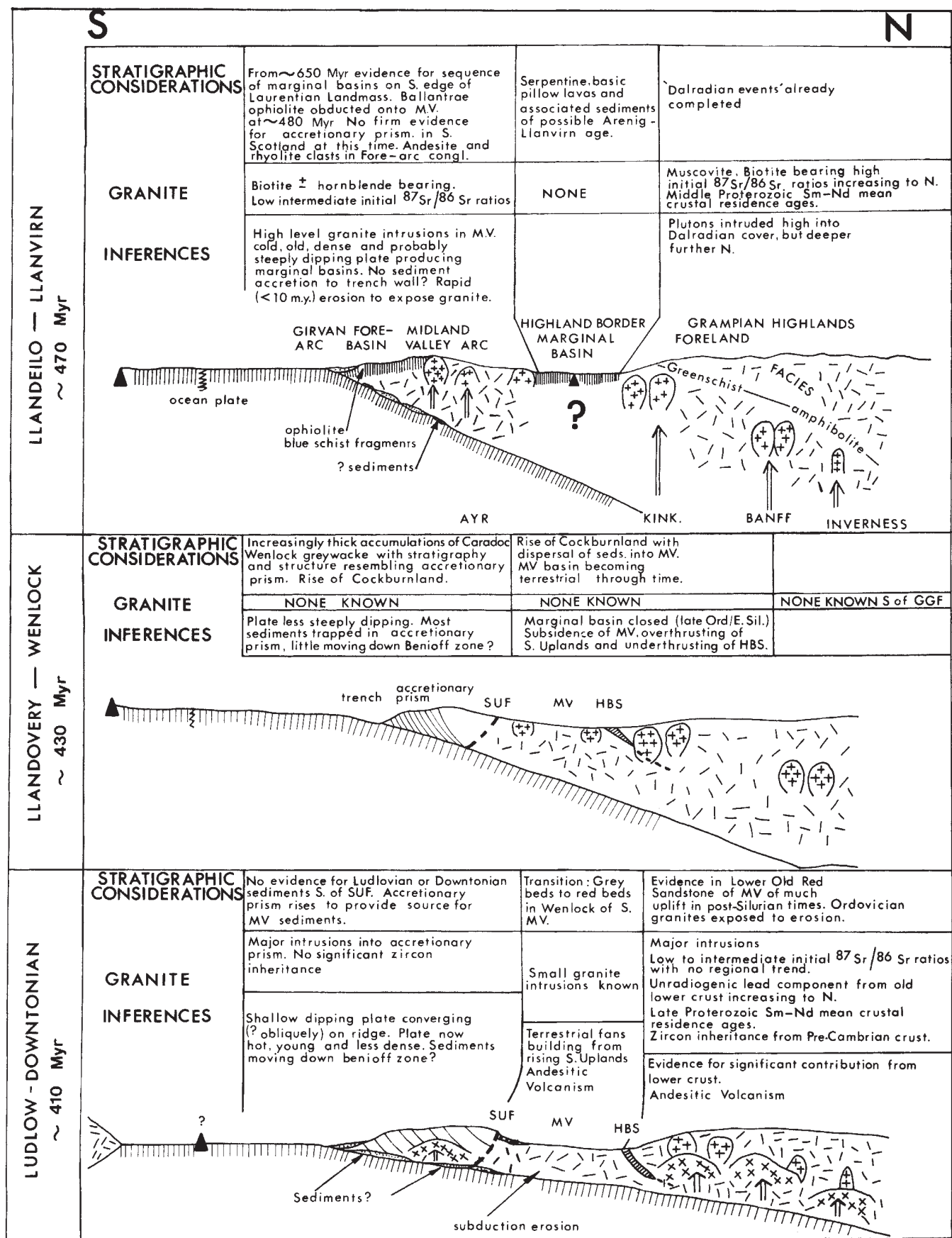


Fig. 3 Reconstruction of the Southern Uplands-Grampian Highlands during Ordovician-Silurian time, showing facts and inferences referred to in text. See Fig. 1: MV, Midland Valley; HBS, Highland Border Series.

associated clast materials in the conglomerates. As with other plutonic bodies and as suggested by the clast profile, convergence of the margins downwards would result in a balloon shaped plutonic mass¹⁰, which might almost disappear as the land surface was eroded down.

Granite clasts dated¹¹ at ~470 Myr and associated with an influx of andesitic fragments¹², both derived from the southern Midland Valley, have been related to the late Ordovician two-mica granites in the Dalradian of Banff and Aberdeenshire. In the latter region ages range from 475 ± 5 Myr for the Stichen Granite (U-Pb on monazite¹³) to 453 ± 4 Myr and 444 ± 9 Myr for the Kennethmont and Aberchirder granites (Rb-Sr whole rock isochrons⁹). A regional suite of tourmaline-bearing pegmatites have been dated at 463 ± 6 Myr (Rb-Sr muscovite isochrons^{14,15}). All these intrusions are post-tectonic. Further north-west, in the Moine schists below the Dalradian metasediments, late tectonic pegmatites and a small two-mica granite (the Glen Kyllachy Complex) have been dated at 445 ± 5 Myr and ~443 Myr (Rb-Sr muscovite and whole rock isochron ages⁵). If the Rb-Sr isotope data are taken alone, there seems to be a ~30 Myr, decreasing age trend from the south-east to the north-west away from the trench. The Stichen Granite in north-east Aberdeenshire with a reliable U-Pb monazite age of 475 ± 5 Myr seems to be older. Even so, the age of this pluton falls within the ~35 Myr range of late Ordovician granite emplacement.

Discussion

As the Ordovician granites extend south-east of the Highland Boundary Fault where there is no evidence for the Grampian orogenic event, this suite has been interpreted in terms of being related to subduction¹¹. Yet high initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and various geochemical features have been presented as evidence that the two-mica granites of north-east Aberdeenshire and the Glen Kyllachy complex have been derived from metasediments^{5,9,16}. The new data from the denuded granites along the Highland Boundary Fault provide evidence for a common origin of all the middle to late Ordovician granites and andesites and a link between subduction and high level melting.

The ~460 Myr granites are clearly younger than those migmatites north of the Highland Boundary Fault which are of ~510 Myr Grampian age¹⁷ or older¹⁸. In view of their proximity to the Highland Boundary Fault it would be difficult to envision their generation through burial metamorphism of Grampian age¹⁹. Also these granites slightly predate the Caradocian marginal basin closure along the Highland Boundary Fault (Fig. 3, see ref. 20). Their generation and rise past the present erosion level almost certainly required a subcrustal heat source.

Burnham²¹ has shown that mantle-derived basic magma can produce extensive partial melting and assimilation of hydrous rocks of felsic composition. Such a process is analogous to the mechanism of magma ascent and country assimilation as developed by Bowen^{22,23}. Thus the rise of a magma continues while a net balance of heat is maintained from that lost by melting and or assimilation of country rocks and gained by the latent heat of crystallization of a more basic residuum. The assimilation crystal fractionation process is not unique to the original magma and as the magma bores its way upwards, the major element chemistry will approach that of a partial melt of the country rocks.

Initial Sr isotopic ratios for all the late Ordovician granites are plotted in Fig. 2. Ratios from each area are presented as cumulative frequency plots for increasing values and the north-west is represented by the right-hand side of the diagram. The granites show a systematic increase in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios as one passes from the Ordovician trench to the interior of the continent. The age and initial ratio pattern of the late Ordovician granites thus seems to be systematic. Although the effects of ultrametamorphism may have been significant, it is reasonable to conclude that the process which relates all these granites, on both sides of a marginal basin, is subduction. The north-westwards increasing $^{87}\text{Sr}/^{86}\text{Sr}$ ratios could then be attributed to

the increasing thickness of older continental crust above the Benioff zone²⁴.

Clearly the high temperatures of metamorphic rocks in a post-tectonic setting when geotherms are raised in the early stages of uplift²⁵ should by the above heat balance mechanism allow the magmas to rise high into the upper crust as well as increase the proportion of melt derived from the upper crust. Also, in view of the westward orogenic diachronism across the Grampian region⁵ magmatism may have persisted later in the north-west. In the latter region earlier magmatism may be represented by late tectonic 'discordant migmatite'²⁶. Also the apparent westward migration of plutonism is probably partly the result of the time constant of subduction.

Variations in the nature and extent of plutonic and volcanic activity have been related to differences in characteristics of the ocean plate being consumed at the trench²⁷⁻²⁹. In Recent to Jurassic examples, where adjacent ocean lineation patterns allow reconstructions of this type, the relationship between characteristics of ocean plate and plutonic activity is still equivocal³⁰. In the Caledonian cycle, changes in the characteristics of the Iapetus plate can only be inferred from the stratigraphical record in southern Scotland. Criteria for the recognition of these changes are given in Fig. 3. Vlaar and Wortel³¹ and Molnar and Atwater³² have shown that a relationship exists between angle of subduction and age of plate—older plates are thicker, colder and denser and therefore dip more steeply than the younger. These authors also suggest that island arcs and accompanied marginal basins are produced by the steeply dipping subduction zones.

The geological record in southern Scotland suggests the presence of volcanic arcs and marginal basins in Arenig to Llandeilo times³³ together with possibly older blue schist, from which it might be inferred that the Iapetus plate was cold and steeply dipping. It was during this interval that the early granites (discussed here) were emplaced. These are high level, relatively low volume granites showing much upper crustal involvement. At this same time, despite evidence for rapid uplift in the Midland Valley source¹¹, the volume of trench accretion does not seem to have been substantial³⁴. Some of this sediment may have been consumed in the subduction zone.

In the interval Caradoc–Ashgill, the beginning of sedimentary accretion coincides with decreasing granite magmatism moving away from the trench. The following Llandovery and Wenlock stages are typified by a major accretionary prism and during this

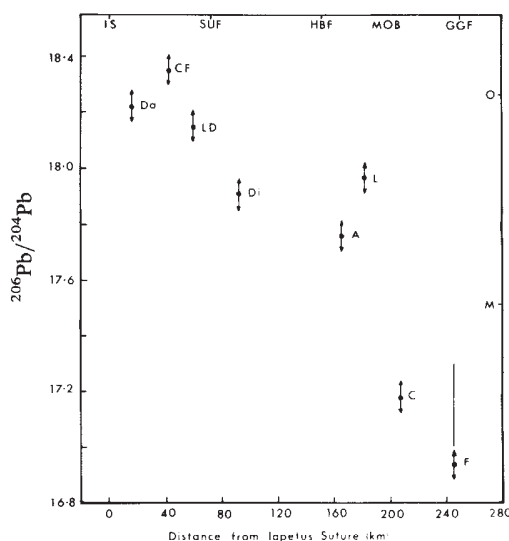


Fig. 4 Plot of age corrected $^{206}\text{Pb}/^{204}\text{Pb}$ ratios for feldspar separates from Silurian–Devonian granites of Scotland⁴⁵ versus distance from the Iapetus Suture. See Fig. 1. Doe and Zartman's⁴⁷ mantle and 'orogene' values of $^{206}\text{Pb}/^{204}\text{Pb}$ 400 Myr ago are represented by M and O. These authors estimate Upper and Lower crustal values at 18.71 and 16.67 respectively. Lewisian rocks yield values in the range 13.41–16.91 (ref. 48).

time, no granite intrusions have been recorded south of the Great Glen Fault. Abundant calc-alkaline plutonism at this time north of the Great Glen Fault can be explained in terms of separate subduction histories before the opposing Highland regions were juxtaposed by sinistral movements along this transcurrent fault^{5,35}.

The next period of major plutonism south of the Great Glen Fault took place between ~415 and 390 Myr. (Fig. 1)^{13,36-39}. There is no record of any sediment accretions at this time in the south of Scotland. Although there may be many reasons for this absence of sediments north of the Iapetus suture, it is possible that they were taken down the subduction zone as in the former period of major plutonic activity. Indeed, the patterns of granite plutonism and sedimentary accretion observed in California⁴⁰, Alaska⁴¹ and Scotland support continuous crustal recycling^{42,43}.

At this stage it is possible that together with the sediments on the ocean floor, part of the accretionary prism was also abraded into the subduction zone. A hypothesis of a mixed derivation of sedimentary and mantle material to make up the voluminous Silurian-Devonian granites is consistent with the Sr (ref. 37) and O isotope data^{38,44}. However, common lead isotope data from feldspars show that lower crustal materials were also involved in the formation of these granites⁴⁵. As lower crustal rocks generally have low ⁸⁷Sr/⁸⁶Sr ratios, the absence of a geographical trend in this parameter (Fig. 2) is not diagnostic. The common lead isotope evidence is supported, however, by more recent Sm-Nd isotope evidence⁴⁶.

With the separate history of the later granites on either side of the Great Glen Fault, the Pb isotope trend⁴⁵ for the granites to the south can be shown to be surprisingly consistent. On Fig. 4 the ²⁰⁶Pb/²⁰⁴Pb ratios of the later granites south-east of the

Great Glen Fault have been plotted against distance from the Iapetus suture. More data would be desirable. However, note that four ²⁰⁶Pb/²⁰⁴Pb ratios from the Foyers complex (in the range 17.0-17.3) reported by Pankhurst³⁹ and showing the internal variability in a single pluton are consistent with the trend of northwestwardly decreasing ratios. The consistent downward trend of this ratio away from the Iapetus suture is best interpreted in terms of an increasingly unradiogenic (Pb) lower crustal input into the granites. In view of the relative Pb concentrations of upper and lower crustal rocks, only limited amounts of upper crustal rocks are likely to have been assimilated⁴⁷.

The trend of upper crustal (radiogenic Sr) involvement of the Ordovician granites seems to have been repeated with respect to the lower crust (unradiogenic Pb) in the case of the Silurian-Devonian granites. This feature can be partly explained by the large amount of Highland uplift, erosion and cooling which occurred in the intervening (Silurian) time. At the same time, the greater volume of the later granites, which may have swamped the upper crustal contribution, argues for high temperatures in the lower crust and the mantle⁴⁸. The voluminous nature of these granites combined with the abundance of andesitic volcanism, often directly relatable to the plutons, suggests that in the final stages of closure of Iapetus the continent over-rode comparatively young, hot ocean floor at a low angle of subduction. This interpretation is also consistent with the absence of island arcs in the Silurian.

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Expression in *E. coli* of maize and wheat chloroplast genes for large subunit of ribulose biphosphate carboxylase

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Synthesis of the large subunit polypeptide of ribulose biphosphate carboxylase can be detected in Escherichia coli cells containing the chloroplast genes from maize and wheat. The chloroplast DNA sequences contain a 'promoter' that, in Escherichia coli, initiates transcription and translation of the genes. This synthesis of large subunits has been substantially increased using the powerful λ promoter, P_L , to drive transcription of the maize gene.

ALTHOUGH much effort has recently been devoted to constructing plasmids in which the expression of mammalian genes can be directed from prokaryotic promoters¹⁻⁷, there have been no reports of the expression of plant genes in *Escherichia coli*. Chloroplast genes from higher plants would seem to be particularly favourable candidates for expression in bacteria because of the similarities already established between chloroplasts and prokaryotic organisms. Thus, in both there are 70S ribosomes containing 23S, 16S and 5S rRNA⁸, there is strong sequence homology between maize chloroplast and *E. coli* 16S rRNA genes⁹ and *N*-formylmethionyl-tRNA is used for the initiation of polypeptide chain synthesis¹⁰. Furthermore, chloroplast genes have been transcribed and translated successfully *in vitro* using *E. coli* cell-free extracts¹¹. Accordingly, we have investigated chloroplast gene expression in bacteria using the maize and wheat genes for the large subunit polypeptide of ribulose biphosphate carboxylase (RuBPCase).

Our choice of this gene was favoured by the extensive knowledge of both the structure and function of the gene and the enzyme. RuBPCase [3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39] holoenzyme is a major soluble protein in green plants and fixes CO₂ in the photosynthetic pentose phosphate pathway¹². It is present in the stroma of chloroplasts as an oligomer with 16 subunits and a molecular weight (MW) of ~560,000 (ref. 13). Eight of these subunits are small (MW 12–18,000) and the remaining eight are large (MW 50–55,000). The large subunit contains the catalytic sites for the carboxylase and oxygenase reactions¹² but the function of the small subunit remains undetermined. The small subunits are nucleus encoded¹⁴ and synthesized on cytoplasmic ribosomes as a precursor which passes through the chloroplast membrane and is processed to its final size^{15,16}. It is assembled with large subunits that are encoded by chloroplast DNA¹⁷ and synthesized on chloroplast ribosomes¹⁸. The DNA sequence of the maize large subunit gene¹⁹ and a pea small subunit cDNA clone²⁰ have been established.

We describe here the synthesis of the large subunit polypeptide of RuBPCase in *E. coli*, apparently under the control of chloroplast sequences that function as promoters in this

bacterium. The synthesis of the plant protein can also be manipulated using promoters from bacteriophage λ during lytic infection. The demonstration that chloroplast genes from higher plants are expressed in *E. coli* provides many new opportunities in plant and bacterial molecular biology.

Construction of λ and plasmid molecules containing RuBPCase large subunit gene sequences

The chloroplast chromosome of all characterized species contains a single copy of a gene for the large subunit of RuBPCase (RuBPCase LS), and the position of this gene has been mapped for maize²¹ and wheat (C. M. Bowman, B. Koller, H. Delius and T. A. Dyer, personal communication). The gene from both species can be isolated intact on a fragment generated by digestion with the restriction endonuclease *Bam*HI; 4.3 kilobase pairs for maize and 9.7 for wheat. Both these fragments have been cloned directly into the *Bam*HI site of pBR322. The maize fragment has the additional advantage of being a suitable size for cloning into the *Bam*HI λ insertion vector NM570-BV2 (ref. 22). Our approach in the construction of λ and plasmid recombinants containing the RuBPCase LS sequences is detailed in Fig. 1 legend.

The orientation of the maize gene in λ was determined using the asymmetric location of *Eco*RI sites within the chloroplast DNA fragment²¹ and the phage vector²². Clones were identified in which the RuBPCase LS gene is transcribed either from the *l*-strand of the phage chromosome using the promoter P_L(λ ZmB1'), or from the *r*-strand using the promoter P'_R(λ ZmB1'). The maize gene was transferred to the *Bam*HI site of pBR322 and the two possible orientations (pZmB1A and pZmB1B) distinguished by the asymmetric location of *Pst*I sites within the chloroplast DNA fragment²¹ and the plasmid²³. The plasmid pTac39 contains the wheat RuBPCase large subunit gene on a fragment inserted into the *Bam*HI site of pBR322 and we derived the plasmid pTac518 from pTac39 by reversing the orientation of the chloroplast fragment (Fig. 1).

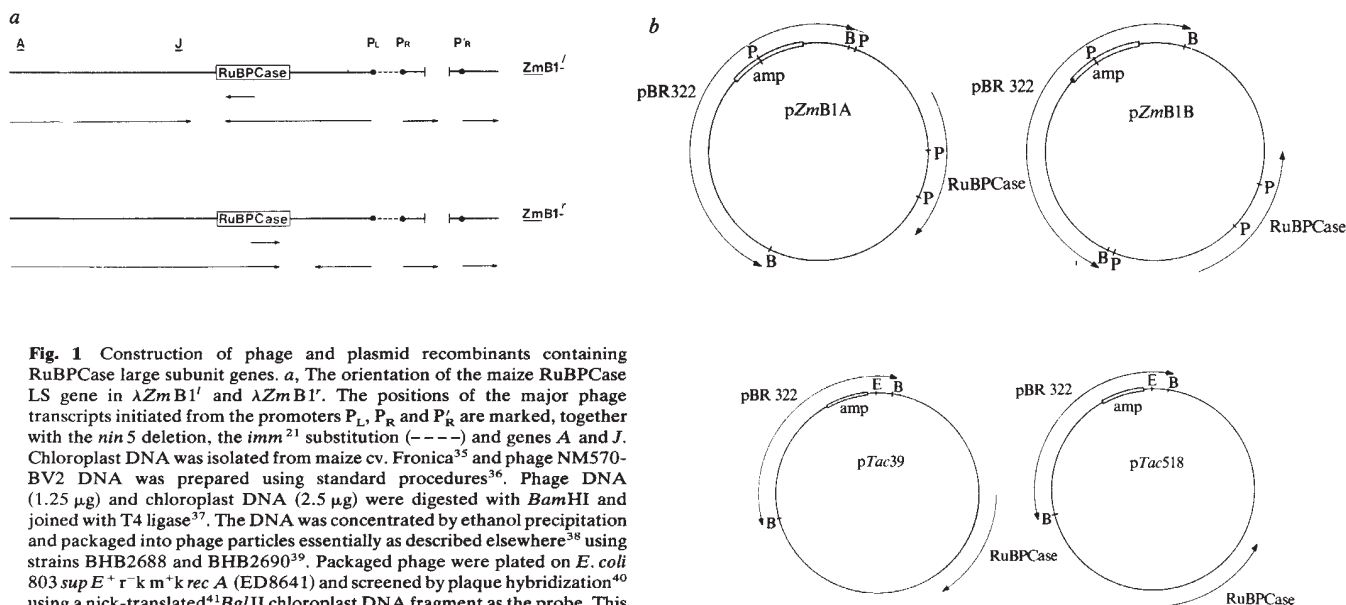


Fig. 1 Construction of phage and plasmid recombinants containing RuBPCase large subunit genes. *a*, The orientation of the maize RuBPCase LS gene in λ ZmB1' and λ ZmB1'. The positions of the major phage transcripts initiated from the promoters P_L, P_R and P'_R are marked, together with the *nin* 5' deletion, the *imm* 21 substitution (---) and genes A and J. Chloroplast DNA was isolated from maize cv. Fronica³⁵ and phage NM570-BV2 DNA was prepared using standard procedures³⁶. Phage DNA (1.25 μ g) and chloroplast DNA (2.5 μ g) were digested with *Bam*HI and joined with T4 ligase³⁷. The DNA was concentrated by ethanol precipitation and packaged into phage particles essentially as described elsewhere³⁸ using strains BHB2688 and BHB2690³⁹. Packaged phage were plated on *E. coli* 803 sup E⁺ r⁺ k⁺ m⁺ k⁺ rec A (ED8641) and screened by plaque hybridization⁴⁰ using a nick-translated⁴¹ *Bgl*II chloroplast DNA fragment as the probe. This fragment contains the complete RuBPCase LS gene sequence²¹. Positive clones were plaque purified and phage isolated from liquid lysates³⁶. The orientation of the RuBPCase LS gene in the phage chromosome was deduced from the asymmetric distribution of *Eco*RI sites^{21,22} and confirmed by transfer to nitrocellulose filters⁴² and hybridization with the nick-translated *Bgl*II fragment. *b*, The orientation of the maize RuBPCase LS gene in plasmids pZmB1A and pZmB1B. The position of *Bam*HI (B) and *Pst*I (P) sites and the direction of transcription of the maize gene are indicated. λ ZmB1 DNA (2 μ g) and pBR322 DNA (0.2 μ g) were digested with *Bam*HI and joined with T4 ligase. The DNA was transformed into strain ED8641 and clones resistant to ampicillin and sensitive to tetracycline selected. Purified plasmids⁴³ with the RuBPCase LS gene in either of the two orientations were identified by the asymmetric distribution of *Pst*I sites^{21,23} and confirmed by hybridization with the *Bgl*II fragment as described in *a*. *c*, The orientation of the wheat RuBPCase LS gene in plasmids pTac39 and pTac518. The position of *Bam*HI (B) sites and the direction of transcription of the wheat gene are indicated. The map of pTac39 was produced from information provided by C. M. Bowman and T. A. Dyer. The wheat fragment was excised with *Bam*HI and re-joined with T4 ligase to obtain pTac518 with the fragment in the opposite orientation.

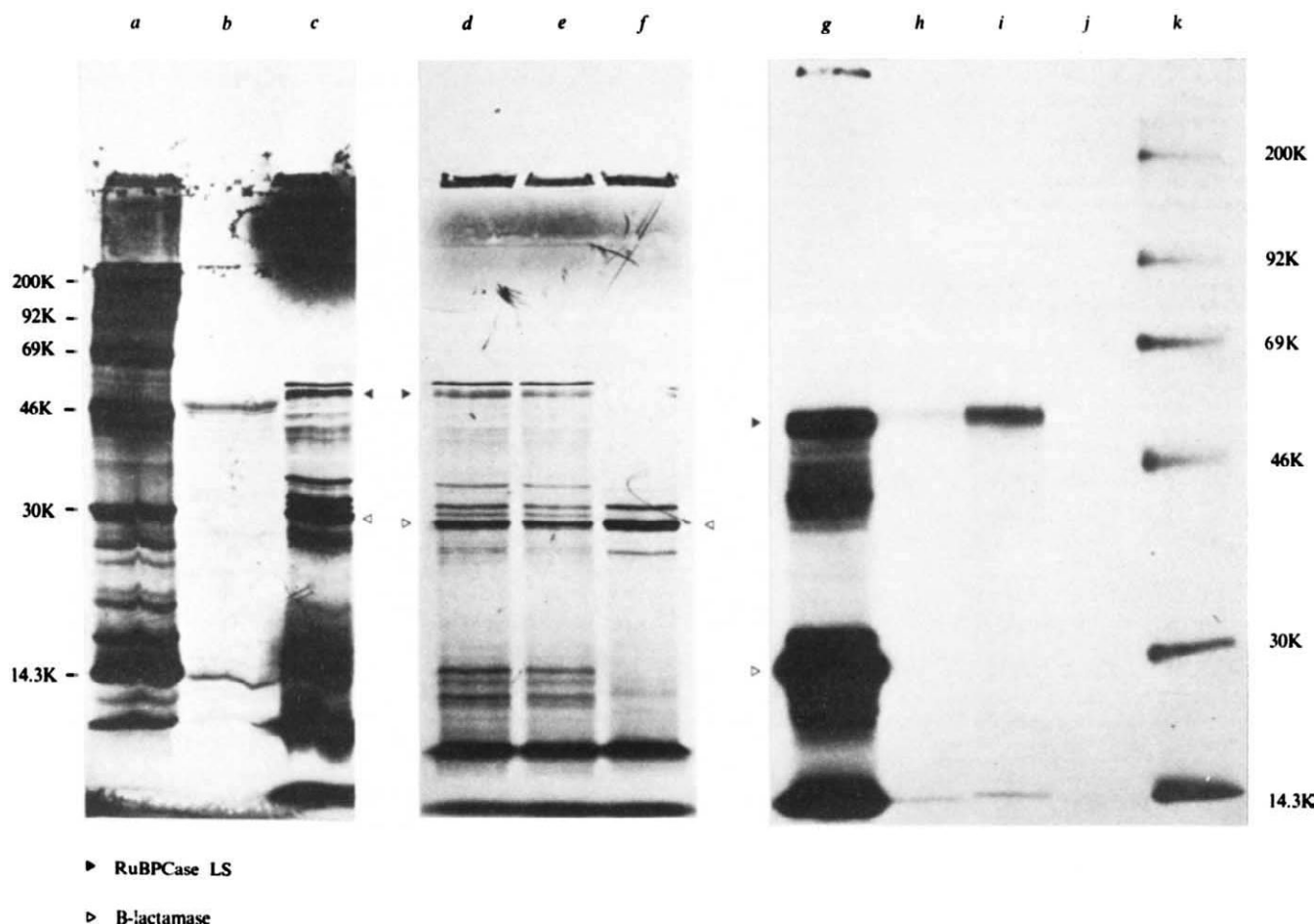


Fig. 2 Expression of RuBPCase LS in *E. coli* minicells. The plasmids pBR322, pTac 39, pTac 518, pZm B1A and pZm B1B were transformed into the minicell strain DS410²⁴. Minicells were prepared from 200 ml of an overnight culture grown in brain heart infusion²⁴. The minicells were preincubated for 30 min at 37 °C in minimal medium and then incubated for a further 60 min in the presence of 10–20 μ Ci L-[³⁵S] methionine (Amersham). Prewarmed L broth was added and after a further incubation for 15 min the minicells were collected by centrifugation and lysed by boiling in 100 μ l of 2% (w/v) SDS for 2 min. The number of proteins synthesized was examined by SDS-polyacrylamide gel electrophoresis⁴⁴, followed by autoradiography of the dried gels. For immunoprecipitation the SDS minicell lysate was diluted with 1 ml of Triton buffer (50 mM Tris-HCl pH 7.8, 0.15 M NaCl, 2 mM EDTA, 1% (v/v) Triton X-100) and clarified at 20,000g for 10 min. To the supernatant was added 10 μ l of either anti-RuBPCase serum or pre-immune serum. After incubation at 20 °C for 2 h the antigen-antibody complex was absorbed to protein A-Sepharose (equivalent to 5 mg dried Sepharose) by shaking gently for 1–2 h. The Sepharose was washed once with Triton buffer containing 1 M NaCl and three times with Triton buffer. The Sepharose pellet was resuspended in 20 μ l of SDS loading buffer and applied directly to a 10% polyacrylamide gel and electrophoresed in the presence of SDS⁴⁴. The dried gels were examined by autoradiography or fluorography²⁹ at –80 °C. Wheat and maize RuBPCase were purified for immunization, and as standards, by chromatography on Sephadex G-75, Ultrogel AcA22 and DEAE-Sephadex A50⁴⁵ followed by sucrose gradient centrifugation⁴⁶. Gels were calibrated with a [¹⁴C]-methylated protein mixture (Amersham, CFA.626). The molecular weight markers were lysozyme (14,300), carbonic anhydrase (30,000), ovalbumin (46,000), bovine serum albumin (69,000), phosphorylase b (92,000) and myosin (200,000). a, Molecular weight markers (long exposure); b, pTac 39 incubated with anti-RuBPCase serum and protein A-Sepharose; c, total pTac 39; d, total pTac 39; e, total pTac 518; f, total pBR322; g, total pZm B1A; h, pZm B1A incubated with a RuBPCase immunoabsorbent and eluted with 8 M urea⁴⁵; i, pZm B1A incubated with anti-RuBPCase serum and protein A-Sepharose; j, pZm B1A incubated with pre-immune serum and protein A-Sepharose; k, molecular weight markers (short exposure).

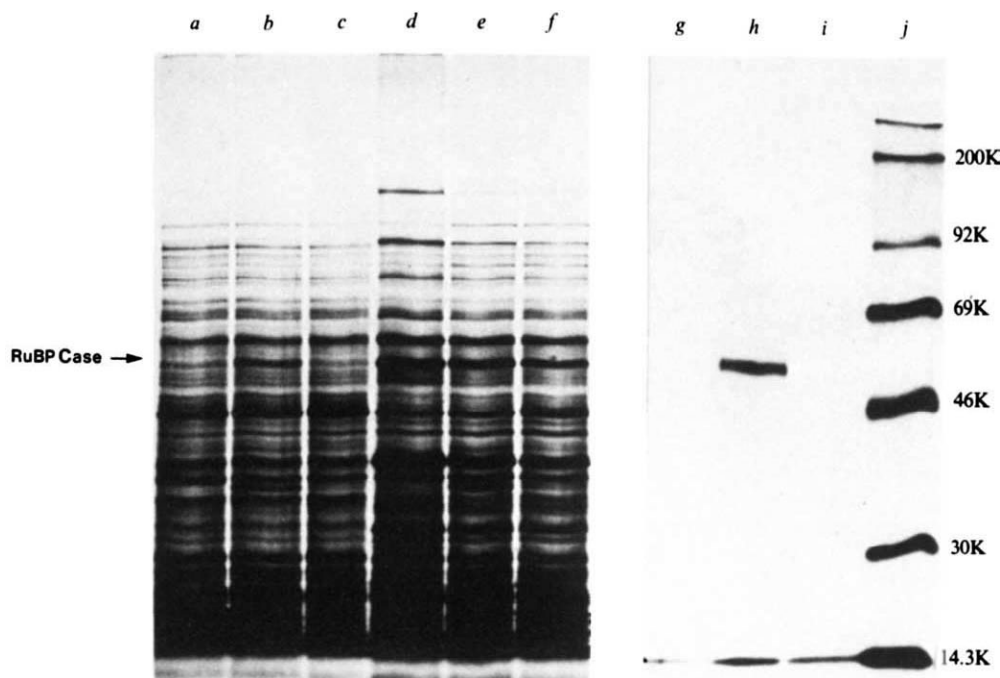
***In vivo* expression of RuBPCase large subunit gene on plasmids**

Plasmids with both orientations of the RuBPCase LS gene from maize (pZm B1A and pZm B1B) and wheat (pTac 39 and pTac 518) were transformed into the minicell-producing strain DS410²⁴. Minicells containing each of the four plasmids and a pBR322 control were prepared and incubated in the presence of L-[³⁵S] methionine to label specifically proteins synthesized from the plasmids²⁴. Autoradiography of extracts separated on SDS-polyacrylamide gels demonstrated that several additional polypeptides were synthesized when the recombinant plasmids were present in the minicells, compared with the pBR322 control (Fig. 2). One of these additional polypeptides had a MW of 52–53,000 and a similar mobility to the purified RuBPCase large subunit isolated from leaves (data not shown). The identity of this polypeptide was confirmed by incubating the *in vivo*

labelled minicell extracts with anti-RuBPCase serum and recovering the antigen-antibody complex by absorption to protein A-Sepharose. Radioactive polypeptides eluted from the protein A-Sepharose were resolved by SDS-polyacrylamide gel electrophoresis mainly as a major band of MW 52–53,000, although traces of other polypeptides could be detected after prolonged fluorography (Fig. 2). The RuBPCase LS polypeptide was not recovered if pre-immune serum was incubated with the minicell extracts. The slight difference in mobility between the immunoabsorbed wheat polypeptide and the corresponding polypeptide in the total minicell extract (Fig. 2, tracks b, c) is probably due to distortion in the gel by an unlabelled polypeptide present in large amounts in this part of the gel, which we suspect to be the heavy chain of IgG that is absorbed to the protein A-Sepharose.

The number and relative amounts of polypeptides synthesized from the RuBPCase LS plasmids were independent of the orientation of the chloroplast fragments in pBR322. Tran-

Fig. 3 Expression of maize RuBPCase LS during lytic infection with phage recombinants. *E. coli* S159 was grown in minimal medium with maltose and UV light-irradiated essentially as described by Jaskunas *et al.*²⁵. 50 μ l (5×10^7 bacteria) were mixed on ice with 5 μ l (1×10^8 plaque-forming units) of phage suspension. For cells labelled early during infection the samples were incubated at 37 °C for 3 min and then diluted with 200 μ l of prewarmed M9 salts, 1 mM MgSO₄, 0.3% (w/v) maltose, 0.04 μ g methionine per ml, containing 10 μ Ci of L-[³⁵S]methionine. After 10 min the cells were chased with 30 μ l of 2 mg ml⁻¹ methionine for 1 min. For cells labelled late during infection the samples were incubated at 37 °C for 12 min, diluted with the isotope solutions as above and incubated for 10 min, and finally chased as before. Protein synthesis was terminated by the addition of 1 ml of cold acetone at -20 °C for 30 min. Precipitated proteins were collected by centrifugation and dried. Total protein synthesis was examined by dissolving the pellet in SDS loading buffer, resolving the polypeptides by electrophoresis on 10% polyacrylamide gels⁴⁴ and autoradiography of the dried gels. For immunoabsorption the precipitated protein was boiled for 2 min in 100 μ l of 2% (w/v) SDS, cooled to room temperature and diluted with 1 ml of Triton buffer (Fig. 2). Conditions for incubating with antiserum and protein A-Sepharose were identical to those for Fig. 2. The immunoabsorbed protein was detected by fluorography²⁸ at -80 °C. Early labelling with: a, λ NM 570-BV2; b, λ ZmB1'; c, λ ZmB1'. Late labelling with: d, λ NM 570-BV2; e, λ ZmB1'; f, λ ZmB1'. Immunoabsorbed polypeptides after early infection with: g, λ NM 570-BV2; h, λ ZmB1'; i, λ ZmB1'. The MW markers (j) were identical to those of Fig. 2.



scription and translation from sites within the chloroplast fragments seems the most likely explanation of this expression, in view of the absence of an orientation effect on the synthesis of maize and wheat RuBPCase LS. What is known of pBR322 transcription suggests that the level of expression of a gene using external promoters would be very different if inserted in opposite orientations in the *Bam*HI site.

To estimate the proportion of RuBPCase LS synthesized compared with β -lactamase, which is encoded on the same plasmid, we used scanning densitometry of the autoradiographs. After adjustment for the methionine content of RuBPCase LS and β -lactamase, the amount of RuBPCase LS synthesized is equivalent to approximately a 1:3 molar ratio.

Lytic expression of maize RuBPCase LS

To increase the synthesis of the large subunit of maize RuBPCase in *E. coli* we used P_L-initiated transcription to drive expression of the chloroplast gene. Phage-directed protein synthesis was examined by infecting UV light-irradiated S159 cells in the presence of L-[³⁵S]methionine²⁵ and resolving the products by polyacrylamide gel electrophoresis (Fig. 3). Incubation of lysates with anti-RuBPCase serum and immunoabsorption to protein A-Sepharose were used to verify the identity of the large subunit (Fig. 3).

Lytic expression of maize RuBPCase LS is strongly dependent on the orientation of the gene in the phage chromosome and on transcription from λ promoters. The kinetics of synthesis of the polypeptide (Fig. 4) demonstrate that when the chloroplast gene is transcribed from the *l*-strand of λ , RuBPCase LS accumulates rapidly in the first 10 min post-infection and accounts for 0.5–1.0% of total protein synthesis at this stage; RuBPCase LS synthesis then decreases. This pattern of expression is entirely consistent with the synthesis of RuBPCase LS as an early protein by P_L-initiated transcription, followed by repression of P_L by the phage *cro* gene product²⁶. When the

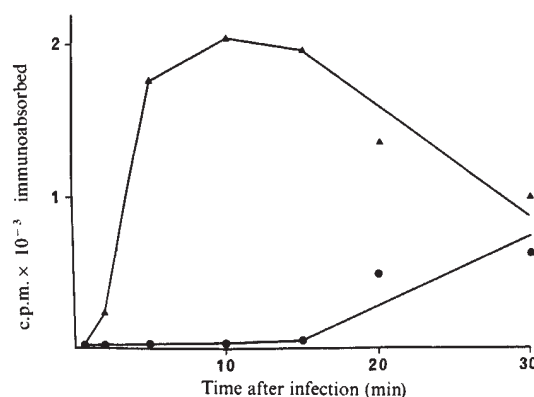


Fig. 4 Kinetics of expression of maize RuBPCase LS gene in *E. coli* cells infected with λ ZmB1' (Δ) or λ ZmB1' ($\bullet$). UV light-irradiated S159 cells were prepared²⁵. 300 μ l (3×10^8 bacteria) were mixed on ice with 30 μ l (6×10^8 plaque-forming units) of phage suspension. After incubation at 37 °C for 3 min, 1.2 ml of prewarmed M9 salts, 1 mM MgSO₄, 0.3% (w/v) maltose, 0.05 μ g L-methionine and 60 μ Ci L-[³⁵S]methionine were added. Duplicate 100- μ l samples were withdrawn at the times indicated and added to 1 ml of cold acetone. After 30 min at -20 °C the proteins were collected by centrifugation, dried and boiled in 100 μ l of 2% (w/v) SDS for 2 min. Triton buffer (1 ml) (Fig. 2) was added and after centrifuging at 20,000g for 10 min, an excess of anti-RuBPCase serum (20 μ l) was added to the supernatant and incubated at 4 °C overnight. The antigen-antibody complex was absorbed to excess protein A-sepharose (equivalent to 10 mg dried Sepharose) by shaking gently for 2 h at 20 °C. The Sepharose was washed three times with 1-ml volumes of 1 M NaCl and once with 0.15 M NaCl, both in Triton buffer. The absorbed protein was eluted with four 250- μ l aliquots of 50 mM Tris-HCl pH 8.0, 8 M urea, 0.2 M NaCl and 100 μ g ml⁻¹ bovine serum albumin. The four aliquots were combined and protein precipitated with an equal volume of cold 10% (w/v) trichloroacetic acid. The precipitates were collected on GF/C filters, washed three times with 5% (w/v) trichloroacetic acid, once with ethanol and dried. The filters were processed by liquid scintillation counting. The results were corrected for background by using S159 cells infected with the vector NM570-BV2 in the presence of L-[³⁵S]methionine and processed as above.

orientation of the chloroplast gene is reversed so that phage-initiated transcription is on the *r*-strand, the pattern of synthesis follows that expected of a late phage protein²⁷ and occurs after 15–20 min post-infection. The maize chloroplast fragment was cloned into the sites *sbam*λ-2 and *sbam*λ-3 (ref. 22), thus eliminating the phage *b* region and its transcription termination sites. This explains the observed transcription of the chloroplast gene from phage promoters regardless of the orientation of the gene.

To confirm the function of the phage promoters on the enhanced expression of the maize RuBPCase LS gene we infected a homoimmune λ lysogen, S159(*imm*²¹), with λZmB1' and λZmB1'. The lysogen synthesizes phage repressor to block the major leftward and rightward transcriptions²⁸ during infection with the λ recombinants, thus allowing the effect of phage transcription on synthesis of RuBPCase LS to be determined. The fact that we were unable to detect any synthesis of RuBPCase LS by fluorography²⁹ of immunoabsorbed extracts from the infected lysogens indicates that the enhanced expression of the chloroplast gene is dependent on λ transcription. Expression of the RuBPCase gene in infected lysogens using the endogenous chloroplast promoter was also not detectable, probably due to the low copy (two) of the template introduced during infection.

Discussion

We have demonstrated that the maize and wheat chloroplast genes for the large subunit of RuBPCase are expressed in *E. coli* to give a product of the correct size. This expression is apparently directed on plasmids by transcription initiated from chloroplast sequences that function as promoters. The maize RuBPCase LS gene and flanking regions have been sequenced and were reported to lack a 'classical' prokaryotic promoter sequence¹⁹. However, scrutiny of the published sequence¹⁹ does reveal structural features similar to those observed in prokaryotic promoters. The sequences of special interest lie between –80 and –125 nucleotides before the start of the codon for the N-terminal methionine residue. The region includes the sequence TTGATA (base pairs –119 to –114) which has a high degree of homology with the conserved TTGACA sequence frequently found (around –35 base pairs) before the initiation point of mRNA transcription in prokaryotes³⁰. Such a sequence is implicated in the initial recognition of the promoter site by *E. coli* RNA polymerase³⁰. This sequence in maize is followed by the sequence TAGATT (base pairs –92 to –87) in a similar position and has significant homology to a Pribnow box³¹. Additional regions of less conserved homology are present on either side of the putative Pribnow box and the TTGATA sequence in maize when compared with a prototype prokaryotic promoter sequence³⁰. A typical prokaryotic ribo-

some binding site GGAGG⁹ is centred at –8 base pairs before the N-terminal methionine codon. Koller and Dyer (personal communication) have demonstrated by electron microscopy that *E. coli* RNA polymerase holoenzyme binds specifically to a region on the maize fragment similar to that possessing the sequences described above.

The maize chloroplast DNA sequence therefore possesses features that would lead one to suspect that expression of this plant gene in *E. coli* was possible and our results confirm this expectation. The amount of RuBPCase LS synthesized compared with β-lactamase indicates that expression of the chloroplast gene from a chloroplast promoter in *E. coli* is not insignificant. The ability of chloroplast sequences to initiate transcription has been investigated further by ligating *Sau* 3A-generated chloroplast fragments from the wheat RuBPCase LS plasmid pTac39 into the *Bam* HI site of the promoter-deleted β-galactosidase plasmid pMC489³² and screening recombinants for the synthesis of the β-galactosidase fragment. Clones have been isolated that synthesize the β-galactosidase fragment when chloroplast sequences are placed at the 5' end of the *E. coli* gene, suggesting that initiation of transcription of β-galactosidase mRNA occurs from a chloroplast promoter sequence³³.

The expression of chloroplast genes in *E. coli*, if a general phenomenon, will be advantageous for several reasons. (1) The synthesis *in vivo* of chloroplast-encoded antigens in bacterial colonies or phage plaques will allow solid-phase radioimmunoassay³⁴ to be used for the rapid identification of clones of interest, avoiding the time-consuming method of *in vitro* coupled transcription–translation systems to identify clones¹¹. (2) It should also make possible the identification of some chloroplast genes by complementation of *E. coli* mutants. (3) *In vitro* and *in vivo* mutagenesis and modification of cloned chloroplast genes can be accomplished and the results of such changes on the expression of the gene and the nature of the product can be monitored in bacterial cells. (4) The use of *E. coli* to synthesize chloroplast proteins will be of value for the purification of proteins present only in small amounts in the plant cell, and in the purification of the subunit components of oligomeric enzymes.

Our demonstration that the level of synthesis of RuBPCase large subunits can be increased by using phage promoters should facilitate examination of the assembly mechanism of the RuBPCase holoenzyme and the catalytic function of isolated large subunits that have not been denatured during preparation.

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Genetic analysis of K88-mediated adhesion of enterotoxigenic *Escherichia coli*

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Four cistrons (*adh*) involved in the expression of the K88 adhesion system have been identified and mapped. Three of these (*adh A*, *adh B* and *adh C*) are located in a single operon (*I*) whereas the fourth (*adh D*) is expressed from a separate promoter (*operon II*). The polypeptides encoded by these cistrons have been identified and their role in the formation and regulation of K88 fimbriae (*pili*) is discussed.

THE ability to adhere specifically to mammalian epithelial cells is an important virulence characteristic of many pathogenic bacteria^{1,2}. Adhesion to host tissues is often the first step in the establishment of an infection³, thus elucidation of the mechanisms promoting adhesion could lead to the development of novel anti-adhesive drugs and improved vaccines. Many enterotoxigenic strains of *Escherichia coli* adhere specifically to the epithelial cells of the host's small intestine, where they secrete enterotoxins which are an important cause of diarrhoea in piglets, calves and human infants⁴⁻⁷.

The adhesiveness of these *E. coli* strains is host specific and often associated with characteristic fimbrial proteinaceous antigens on the bacterial cell surface^{8,9}: K88 and 987P in porcine strains^{10,11}, K99 in bovine strains¹², and the colonizing-factor antigens in human strains¹³. Each of these antigens is encoded by large (~70 kilobase pair) plasmids^{7,13}. An 11.5-kilobase pair DNA fragment encoding the K88 serotype *ac* determinant has been cloned^{14,15} into the multicopy vector plasmid pBR322 (ref. 16). The K88⁺ hybrid plasmid was termed pPS002. To provide conclusive evidence for the role of the K88 antigen in adhesion and to determine the genetic basis of its expression, a number of deletion and transposon Tn5 insertion mutants of pPS002 were isolated and analysed.

Deletion mutants of pPS002 generated *in vitro*

E. coli K12 strain C600 harbouring pPS002 readily agglutinates in anti-K88 sera (K88⁺), causes mannose-resistant haemagglutination (MRHA⁺) of guinea pig erythrocytes, carries K88 fimbriae on its surface and adheres strongly to isolated porcine brush border cells *in vitro* (Adh⁺) (Table 1, Fig. 1). C600 has none of these properties. All the above tests are normally used to identify cells carrying wild-type K88*ac* plasmids.

The smallest deletion of pPS002 that was K88⁺, MRHA⁺, Adh⁺ is pMK005 (Table 1, Fig. 2). This deletion mutant differs from pPS002 in that a 5.0-kilobase pair *Eco*RI fragment (*E*₂) is deleted. The remaining 6.5 kilobase pairs of cloned DNA sequence are sufficient to code for the K88⁺, MRHA⁺, Adh⁺ phenotypes.

The deletion mutants pMK001, pMK002, and pMK006 are K88⁻, MRHA⁻ and Adh⁻. Restriction endonuclease analysis of pMK001 DNA showed that this mutation resulted from a deletion extending from near the *Eco*RI site in pBR322 into the cloned DNA sequences and retains only ~1.6 kilobase pairs of the original cloned fragment (Fig. 2). Mutants pMK002 and pMK006 differ from pMK005 in that pMK002 has lost the 1.73-kilobase pair *Eco*RI fragment *E*₃ and pMK006 has lost an extensive part of it. Thus at least part of the sequences encoding the K88⁺, MRHA⁺, Adh⁺ phenotypes are located within the 1.73-kilobase pair *Eco*RI fragment *E*₃.

To determine whether *E*₃ contains sufficient information to express the K88 antigen, the fragment was cloned separately into the single *Eco*RI site on the cloning vector pAC184 (ref. 17). The resulting hybrid plasmid, pMK007, was K88⁻, MRHA⁻ and Adh⁻ (Table 1). These defects could not be

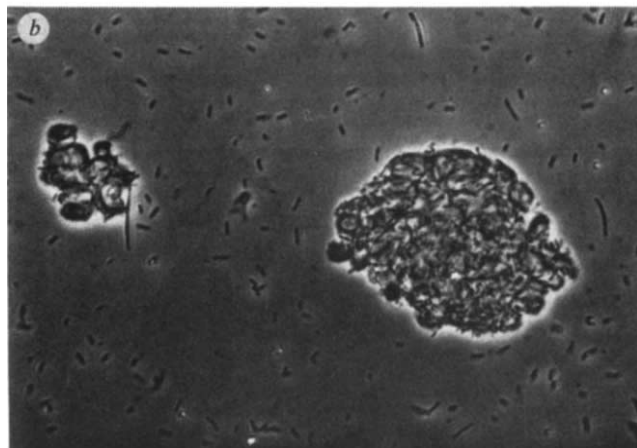
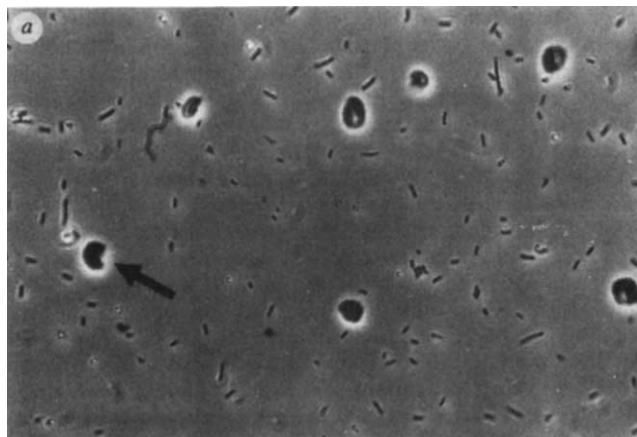
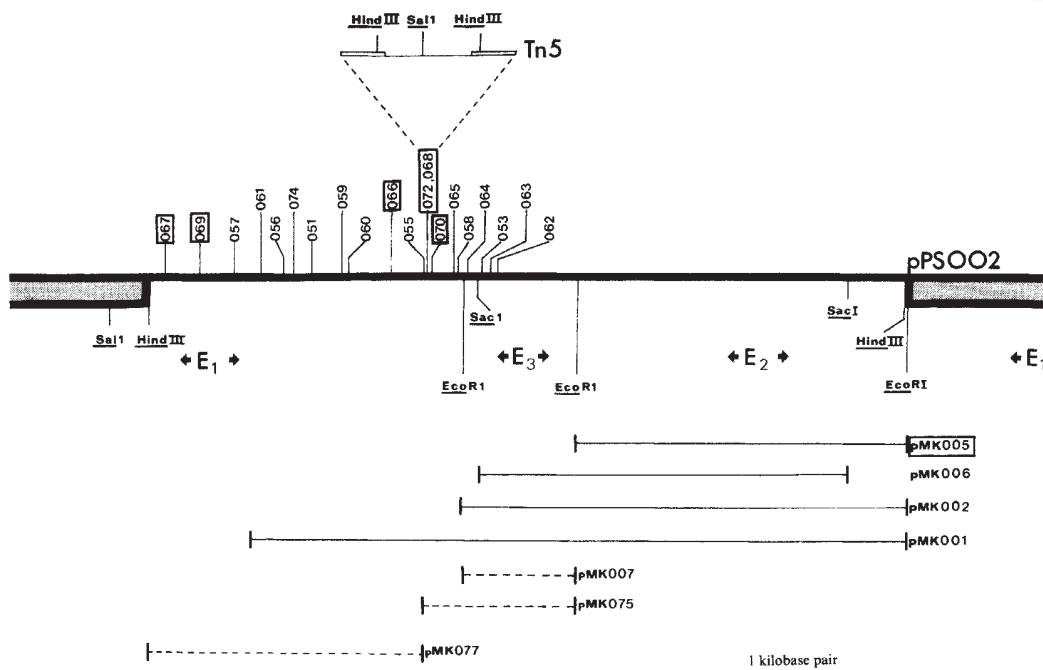


Fig. 1 Adhesion of K88⁺ Adh⁺ strains to porcine brush border cells. The adhesion tests were carried out in the presence of 0.5% (w/v) mannose, as described elsewhere²⁵. Adhesion was scored from - to + + + +. *a*, Pig brush border cells (arrowed) plus the K88⁻ Adh⁻ host strain C600; *b*, pig brush border cells plus C600 harbouring the K88⁺ Adh⁺ plasmid, pPS002.

Fig. 2 Mutants of pPS002. The central solid line represents the cloned DNA sequences in pPS002; the wider shaded boxes at each end represent vector (pBR322) DNA sequences. The cleavage sites for several restriction endonucleases are indicated below the line. The three *EcoRI* fragments are indicated by the letters E₁, E₂ and E₃ in order of decreasing size. The lines beneath correspond to the DNA sequences which have been deleted in the mutants indicated; broken lines correspond to the DNA sequences which have been cloned separately into another vector to generate the hybrid plasmids indicated. The deletion mutant pMK001 was isolated after transforming C600

with linear pPS002 DNA generated by partial cleavage with *EcoRI*. The mutants pMK002, pMK005 and pMK006 were generated by deleting particular restriction enzyme fragments *in vitro*. The hybrid plasmid pMK007 was constructed by cloning *EcoRI* fragment E₃ into the vector pAC184. pMK075 was constructed by recircularizing the largest *HindIII* fragment of the pMK005::Tn5 mutant, pMK070. Cloning of a different *HindIII* fragment from pMK070 into pAC184 generated the hybrid pMK077. In addition to the pPS002 DNA sequences indicated, the hybrid plasmids pMK075 and pMK077 contain 1.16 kilobase pairs of Tn5 DNA sequences. The sites of the Tn5 insertions are indicated by the perpendicular lines above pPS002. The structure of Tn5 is shown above mutant pMK068²⁶. The positions of the Tn5 insertions were mapped by a series of *SalI* and *EcoRI*, *HindIII* double digests. Mutants whose numbers are enclosed in boxes (for example pMK068) are K88⁺Adh⁺; the remaining mutants are K88⁻Adh⁻. Plasmid DNA was isolated as described elsewhere²⁷. The conditions for restriction enzyme digests and the T₄ ligase reactions were as recommended by the manufacturers. Transformation of C600 was carried out as described elsewhere²⁸ and agarose gel electrophoresis as described in ref. 29. The Tn5 insertions into pMK005 were isolated by infecting a Su⁻ host harbouring pMK005 with the phage λ 467 b221 c1₈₅₇ O₂₉ P₈₀ rex::Tn5 at a multiplicity of ~0.5. This phage cannot lysogenize, nor can it replicate in a Su⁻ host. Phage and host were mixed and incubated for 2–3 h at room temperature to allow transposition of Tn5 and expression of the kanamycin resistance (Km^r) genes. Dilutions were plated onto nutrient agar containing kanamycin. The plasmid DNA from ~2,000 pooled Km^r colonies was used to transform C600, selecting for ampicillin resistance Km^r transformants. Only one pMK005::Tn5 transformant from each infection experiment was used for further study.



complimented when pMK007 was introduced into C600 harbouring pMK002, indicating either that a gene or an operon involved in the expression of all three phenotypes spans the *EcoRI* site within the cloned DNA sequences in pMK005.

Tn5 insertion mutants of pMK005

To map in more detail the DNA sequences encoding the K88⁺, MRHA⁺, Adh⁺ phenotypes, a series of independent Tn5 insertion mutants in plasmid pMK005 was isolated and mapped (Fig. 2). Twenty-three insertions within the cloned DNA sequences were chosen for further study (see Table 1); 19 of the pMK005::Tn5 plasmids were phenotypically K88⁻, MRHA⁻ and Adh⁻. These mutations spanned an extensive region (~5.5 kilobase pairs) of the cloned DNA sequences. Five of the Tn5 insertions mapping within the cloned sequences were still K88⁺, MRHA⁺, Adh⁺; two of these (pMK069 and pMK067) mapped close to one end of the sequence whereas three (pMK072, pMK070 and pMK068) were clustered in a small region in the centre. This cluster of K88⁺, MRHA⁺, Adh⁺ Tn5 insertions effectively divides pMK005 into two regions which accommodate K88⁻, MRHA⁻, Adh⁻ Tn5 insertion mutations (see Fig. 2). As insertion of Tn5 into a proximal gene in an operon exerts a strong polar effect (~99%) on the expression of distal genes¹⁸, this suggests that the genes involved in the expression of the K88 determinant are organized into at least two distinct operons.

Cells harbouring one of the insertion mutants, pMK066, were found to be K88⁺ and Adh⁺ but were MRHA⁻. It has been assumed that the reactions involved in the adhesion of K88⁺

enterotoxigenic *E. coli* strains to pig brush border cells and to guinea pig red blood cells are identical but the mutant pMK066 indicates that the situation may be a little more complex.

Polypeptides encoded by pMK005 and its deletion mutants in minicells

The two regions of pMK005 DNA which accommodate Tn5 insertions altering the expression of the K88⁺ Adh⁺ MRHA⁺ phenotypes are large enough to encode for polypeptides of molecular weight (MW) up to at least 138,000. The *E. coli* minicell system^{19,20} was used to detect pMK005-encoded polypeptides (Table 1, Fig. 3). In addition to the vector (pBR322)-encoded β-lactamase polypeptides, the K88⁺ Adh⁺ MRHA⁺ plasmid, pMK005, expressed at least four polypeptides of MWs 70,000, 29,000, 23,500 and 17,000 (Fig. 3a). Minicells harbouring the extensive deletion mutant, pMK001, failed to express any of these four polypeptides (Fig. 3d) indicating that they are encoded within the cloned DNA sequences of pMK005. The 23,500-MW polypeptide which has previously been shown to be the K88 antigen is expressed at a very high rate, forming up to 4% of the total cell protein¹⁴.

The deletion mutants pMK002 and pMK006 expressed high levels of the 70,000, 29,000 and 17,000-MW polypeptides in minicells but failed to produce detectable quantities of the 23,500-MW K88 antigen (Fig. 3b, c). Thus, the cistrons encoding the former three polypeptides are located within the 4.8 kilobase pair cloned DNA sequences retained by pMK002 (Fig. 2) whereas at least part of the DNA sequences required for the expression of the K88 antigen are located within the

Table 1 Characteristics of pPS002, pMK005 and their mutant derivatives

Mutants	Agglutination with anti-K88 serum	MRHA	Adhesion to pig brush border cells <i>in vitro</i>	Polypeptides detected in minicells (MW)			
				70,000	29,000	23,500	17,000
C600 host strain	—	—	—	—	—	—	—
pPS002, pMK005, pMK067	—	—	—	—	—	—	—
pMK069, pMK070, pMK072,	+	+	++++	+	+	+	+
pMK068	—	—	—	—	—	—	—
pMK001	—	—	—	—	—	—	—
pMK002, pMK006, pMK062,	—	—	—	—	—	—	—
pMK063, pMK064, pMK065,	—	—	—	—	—	—	—
pMK058, pMK053	—	—	—	+	+	—	+
pMK055	—	—	—	+	+	—	—
pMK066	+	—	++++	+	—	—(IG+)	—
pMK060, pMK059,	—	—	—	—	—	—	—
pMK051, pMK074, pMK056,	—	—	—	—	—	—(IG+)	—
pMK061, pMK057	—	—	—	—	—	—	—
pMK075	—	—	—	—	—	—	—
pMK077	—	—	—	+	+	—	+
pMK075 + pMK077	+	—	++++	+	+	+	+

Slide agglutination tests using anti-K88 serum were done. Serum was prepared by inoculating rabbits with K88 fimbrial subunit, purified as described by Mooi and de Graff²². The anti-K88 serum was dialysed and concentrated as described in ref. 23 and absorbed against C600 before use. Mannose-resistant haemagglutination (MRHA) of guinea pig erythrocytes was tested in the presence of 0.5% mannose as described in ref. 24. *In vitro* adhesion tests were carried out in the presence of 0.5% mannose as described elsewhere²⁵. The degree of adhesion was scored from — to +++++. IG+ indicates that in these mutants low levels of the 23,500-MW K88 antigen could be detected by the immunoprecipitin gel overlay technique described in Fig. 4 legend.

1.7-kilobase pair *Eco*RI fragment, E₃. Minicells harbouring the pAC184-E₃ hybrid plasmid, pMK007, failed to express the K88 antigen (Fig. 3e).

Polypeptides expressed by pMK005::Tn5 mutants in minicells

In addition to polypeptides expressed from pMK005 DNA sequences, all the pMK005::Tn5 mutants expressed four polypeptides in minicells which are known to be encoded by the transposon²¹. The K88⁺ Adh⁺ MRHA⁺ pMK005::Tn5 mutants (pMK067, pMK068, pMK069, pMK070 and pMK072) expressed high levels of the 70,000, 29,000, 23,500 and 17,000-MW polypeptides described above in minicells (Fig. 3s). Six of the K88[—] Adh[—] MRHA[—] pMK005::Tn5 mutants (pMK053, pMK058, pMK062, pMK064 and pMK065) expressed high

levels of the 70,000, 29,000 and 17,000-MW polypeptides but failed to express detectable levels of the 23,500-MW K88 antigen (Fig. 3g–i, k). If the 23,500-MW polypeptide was being expressed in reduced quantities by these mutants it might not be distinguishable from the 24,000-MW Tn5-encoded neomycin phosphotransferase band on the fluorographs, nor would it be readily detected by Coomassie blue staining of SDS polyacrylamide gels of total cell protein. However, the immunoprecipitin gel overlay technique described in Fig. 4 legend also failed to detect any significant production of the K88 antigen by the six mutants (data not shown). The Tn5 insertions in all six of these mutants map adjacent to each other within a ~650-base pair region which spans the *Eco*RI site separating *Eco*RI fragments E₁ and E₃ (Fig. 2). Thus the position of the cistron (*adh* D) encoding the 23,500-MW K88 antigen can be mapped (Fig. 5). The direction of transcription of this cistron is tentatively suggested by the observation that mutants pMK062 and

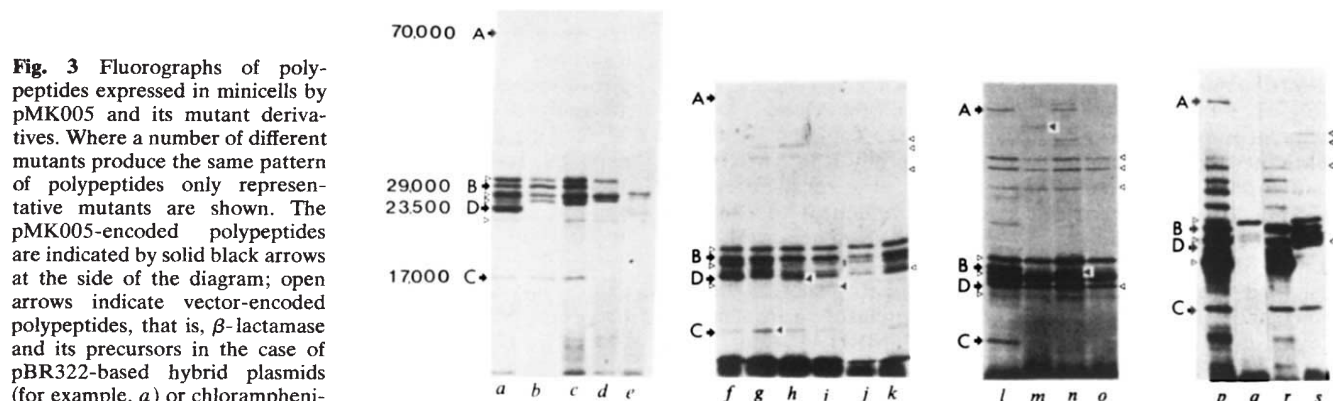


Fig. 3 Fluorographs of polypeptides expressed in minicells by pMK005 and its mutant derivatives. Where a number of different mutants produce the same pattern of polypeptides only representative mutants are shown. The pMK005-encoded polypeptides are indicated by solid black arrows at the side of the diagram; open arrows indicate vector-encoded polypeptides, that is, β -lactamase and its precursors in the case of pBR322-based hybrid plasmids (for example, a) or chloramphenicol acetyltransferase (CAT) in the case of pAC184-based hybrid plasmids (r). Note that in the case of the pAC184-based hybrid plasmid pMK007, the CAT determinant has been insertionally inactivated by the cloned DNA sequence (e). Fragments of polypeptides described in the text are labelled with small solid arrows within the diagram. Plasmid pMK005 and its mutant derivatives were transformed into the *E. coli* minicell strain DS410 (ref. 27). Plasmid-encoded polypeptides were detected by autoradiography and/or fluorography³⁰ of SDS-polyacrylamide gels of ³⁵S-methionine-labelled minicell preparations¹⁹. a, f, pMK005; b, pMK006; c, pMK002; d, pMK001; e, pMK007; g, pMK053; h, pMK062; i, pMK063; (j) pMK055; k, pMK064 (representative of pMK058 and pMK065); l, s, pMK070 (representatives of pMK067, pMK069, pMK072 and pMK068); m, pMK060 (representative of pMK059); n, pMK066; o, pMK051 (representative of pMK056; pMK057; pMK061 and pMK074); p, pMK075 and pMK077; q, pMK075; and r, pMK077.

pMK063 express additional polypeptides of MWs 21,000 and 19,000 respectively, which seem to be fragments of the K88 antigen resulting from termination of transcription or translation by a signal located within the Tn5 insertion. In addition, the 17,000-MW band produced by mutant pMK053 is consistently very intense, suggesting that this is a doublet consisting of a ~17,000-MW fragment of the K88 antigen comigrating with the 17,000-MW polypeptide described above. The sizes of these fragments are consistent with the relative positions of the Tn5 insertions in these mutants.

Mutant pMK055 expressed high levels of the 70,000 and 29,000-MW polypeptides in minicells but failed to produce detectable levels of the 23,500-MW K88 antigen or the 17,000-MW polypeptide (Fig. 3j). The Tn5 insertion in this mutant seems to be located in a cistron (referred to as *adhC*) encoding the 17,000-MW polypeptide. The *adhC* cistron is separated

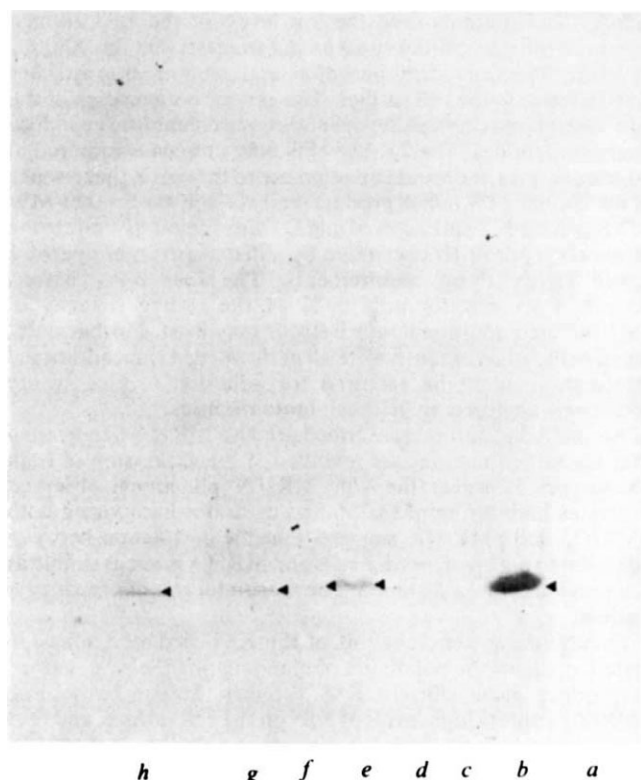


Fig. 4 Immunoprecipitin overlay gel: the method used was a modification of the technique of Broome and Gilbert³¹ (B. Crowe, unpublished results). Whole cells harbouring the plasmid being tested were scraped from a nutrient agar plate and resuspended in SDS-polyacrylamide gel final sample buffer, boiled for 2 min and the polypeptides separated on a 12.5% SDS-polyacrylamide gel. When electrophoresis was complete the gel was washed twice (20 min) with gentle shaking in water and soaked for a further 20 min in 15 mM barbitol-HCl buffer, pH 8.6, containing 1% (v/v) Triton X-100. The gel was transferred to a glass plate and overlaid with a 1% (w/v) agarose gel in barbitol buffer, containing evenly dispersed anti-K88 antibodies. This was incubated overnight at room temperature in a moist box to allow diffusion of polypeptides from the polyacrylamide gel into the agarose anti-K88 overlay gel. Control experiments using the K88⁺ Adh⁺ MRHA⁺ strain, pMK005, showed that the denatured 23,500-MW K88 polypeptide could be readily detected as a precipitin band in the agarose anti-K88 overlay gel. After incubation the immunoprecipitin agarose gel was washed in 0.1 M NaCl and pressed several times to remove excess antibodies and unprecipitated polypeptides. The immunoprecipitin agarose gel was finally dried in a warm air stream and stained with Coomassie blue. a, C600 host; other tracks, C600 harbouring the plasmids b, pMK005; c, pMK077; d, pMK075; e, pMK077 and pMK075; f, pMK057; g, pMK074; h, pMK066.

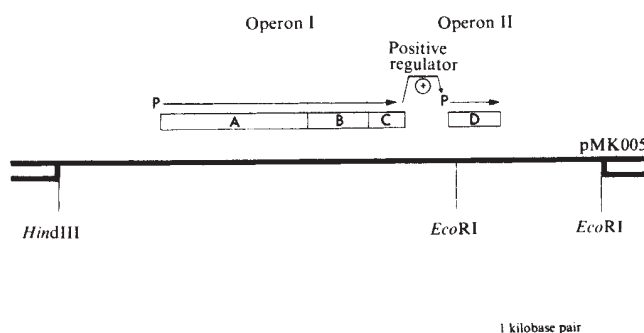


Fig. 5 Model of the genetic organization of the adhesion cistrons. The central solid line represents the cloned DNA sequences in the smallest Adh⁺ K88⁺ MRHA⁺ plasmid, pMK005, the wider shaded boxes at each end represent vector (pBR322) DNA sequences. The boxes above the line represent the *adhA*, *adhB*, *adhC* and *adhD* cistrons; arrowed lines above these indicate the direction of transcription of operons I and II from their respective promoters (P). The 17,000-MW polypeptide product of *adhC* is thought to be a positive regulator required for the expression of operon II (see text).

from the *adhD* cistron by a site which accommodates a number of K88⁺ Adh⁺ MRHA⁺ Tn5 insertions (Fig. 2 and see below). Therefore the absence of the 23,500-MW polypeptide in mutant pMK055 cannot be explained by polarity. It is possible that the 17,000-MW *adhC* product is a positive regulator required for the expression of the *adhD* cistron.

Plasmid pMK066 is the only K88⁺ Adh⁺ MRHA⁻ Tn5 insertion mutant. Minicells containing this plasmid expressed high levels of the 70,000-MW polypeptide but did not produce the other three polypeptides (Fig. 3n). However this mutant produces reduced quantities of the 23,500-MW K88 antigen which was detected by the immunoprecipitin gel overlay technique (Fig. 4h). In addition, pMK066 encodes high levels of a new polypeptide of MW 28,000 (Fig. 3n). We believe that this polypeptide is a fragment of the 29,000-MW polypeptide which is normally expressed from a cistron (termed *adhB*) whose distal end spans the Tn5 insertion site in pMK066. This mutation maps adjacent to the left of *adhC* (Fig. 5). The absence of detectable quantities of the 17,000-MW *adhC* product in minicells harbouring pMK066 is explained by suggesting that *adhB* and *adhC* are located in the same operon (operon I, Fig. 5) and that the *adhB* Tn5 insertion exerts a strong polar effect on the expression of *adhC*. In the absence of normal levels of the *adhC* product, the level of expression of the *adhD* cistron is reduced. In addition to the 28,000-MW fragment of the *adhB* cistron, pMK066 produces a second new polypeptide of MW 31,000, which can sometimes be resolved from the 30,000 β -lactamase precursor on SDS polyacrylamide gels (data not shown). The origin of this polypeptide is not understood.

The Tn5 insertions in the remaining K88⁻ Adh⁻ MRHA⁻ pMK005::Tn5 mutants are located adjacent to each other within a 1.7-kilobase pair region of pMK005 (Fig. 2)—this region is large enough to encode polypeptides totalling a MW of at least 68,000. All these mutants failed to express detectable levels of the four polypeptides in minicells (Fig. 3m, o). Two mutants (pMK059 and pMK060) which map very close together express a new polypeptide of MW 65,000 which could be a fragment of the 70,000-MW polypeptide. The Tn5 insertions in all these mutants seem to be located in a cistron (termed *adhA*) which encodes the 70,000-MW polypeptide. The absence of the 29,000 and 17,000-MW polypeptides is explained by suggesting that the *adhA* cistron is located in the promoter proximal part of operon I (Fig. 5) and *adhA* Tn5 mutants exert a strong polar effect on the expression of the *adhB* and *adhC* cistrons. The polar effects of *adhA* Tn5 mutants and the *adhB* Tn5 mutant indicate that operon I is transcribed from left to right as

described in Fig. 5. The coding capacity of the 2.8 kilobase pairs of DNA extending from the promoter-proximal *adhA*::Tn5 insertion, pMK057, to the promoter-distal *adhC*::Tn5 insertion, pMK055, can be completely accounted for by the *adhA*, *adhB* and *adhC* products (Figs 2, 5).

As the *adhB*::Tn5 mutant, pMK066, expressed low levels of the 23,000-MW K88 antigen in the absence of normal levels of the *adhC* product the *adhA*::Tn5 mutants should also express reduced levels of the *adhD* cistron. Representative *adhA*::Tn5 mutants were analysed by the immunoprecipitin gel overlay technique and were shown to express reduced levels of the K88 antigen (Fig. 4f, g). However, unlike pMK066, these mutants were Adh⁻ and were not readily agglutinated by anti-K88 serum in slide agglutination tests (Table 1). Radial immunodiffusion of concentrated cell-free supernatants of these mutants showed that the K88 antigen was present in the culture supernatants (data not shown). These results suggest that in the absence of the 70,000-MW *adhA* product the K88 antigen is shed into the supernatant rather than being assembled into normal K88 fimbriae.

Cloning operons I and II on different compatible plasmids

If the genetic organization of the *adh* cistrons proposed in Fig. 5 is correct it should be possible to obtain high-level expression of the K88 antigen when operons I and II are present in the same host cell but on different compatible plasmids. Furthermore, the 23,500-MW *adhD* product should only be expressed at high levels when complemented in *trans* by operon I, whereas the *adhA*, *adhB* and *adhC* products should be expressed at high levels even when operon I is present alone in the cell. This hypothesis was tested as follows.

The compatible hybrid plasmids pMK075 (pBR322-operon II) and pMK077 (pAC184-operon I) were produced by cloning *Hind*III fragments from the pMK005::Tn5 mutant, pMK070 (Fig. 2). As expected, cells harbouring either pMK075 or pMK077 alone were K88⁻, Adh⁻ and MRHA⁻ (Table 1) and minicells harbouring pMK075 alone did not produce detectable levels of any of the four polypeptides, whereas minicells harbouring pMK077 alone expressed high levels of the 70,000, 29,000 and 17,000-MW polypeptides only (Fig. 3q, r).

E. coli C600 harbouring both pMK075 and pMK077 expressed reasonably high levels of the K88 antigen (Fig. 4e) and were K88⁺ Adh⁺ but surprisingly MRHA⁻ (Table 1). Minicells harbouring both pMK075 and pMK077 expressed high levels of the 70,000, 29,000 and 17,000-MW polypeptides but low levels of the 23,500-MW K88 antigen and other pMK075-encoded polypeptides (for example, β -lactamase and its precursors; Fig. 3p). It seems that pMK077 and pMK075 do not segregate equally into minicells. However, experiments with whole cells harbouring both of these plasmids (see above) clearly show that pMK075 can express high levels of the 23,500-MW K88 antigen when complemented in *trans* by pMK077. These results support the genetic organization and control of the *adh* cistrons outlined in Fig. 5. However, the MRHA⁻ phenotype of whole cells

harbouring both pMK075 and pMK077 is difficult to understand (see below).

Discussion

Four cistrons, for which we suggested the terms *adhA*, *adhB*, *adhC* and *adhD*, involved in K88-mediated adhesion of enterotoxigenic *E. coli* strains have been identified and mapped (Fig. 5) and their polypeptide products identified in minicells. The 23,500-MW *adhD* product is clearly the K88 fimbrial subunit. The results tentatively suggest the functions of two of the other polypeptides. The 17,000-MW *adhC* product seems to be a positive regulator which controls the expression of *adhD*. However, an alternative explanation to account for the reduction in K88 expression in *adhC*-defective mutants cannot be ruled out. It is possible that in these mutants the *adhD* product has been produced in a precursor form which was not identified by the methods used and that the defect is one of polypeptide processing rather than genetic regulation. The observation that *adhA*::Tn5 mutants shed the low levels of the K88 antigen produced into the culture supernatant suggests that the 70,000-MW *adhA* product forms part of a basal structure that attaches K88 fimbriae to the cell surface. The genetic organization of the *adh* cistrons may reflect the cellular requirement for the individual polypeptides. The 23,000-MW K88 antigen is required in large quantities and would be of no use to the cell in the absence of the 70,000-MW *adhA* product (and possibly the 29,000-MW *adhB* product). Expression of *adhD* from a separate and strong promoter (operon II) controlled by a distal cistron of operon I would satisfy these requirements. The four *adh* cistrons described account for only 54% of the coding capacity of pMK005 and additional *adh* cistrons may exist. Furthermore, because the adhesion tests were all performed *in vitro*, additional information might be required for adhesion *in vivo* where conditions are likely to be much more rigorous.

All the Adh⁻ mutants described are also MRHA⁻, indicating that the same functions are involved in the expression of both phenotypes. However, the Adh⁺ MRHA⁻ phenotype, observed in strains harbouring pMK066 and in strains harbouring both pMK075 and pMK077, suggests that the correlation between adhesion to pig brush border cells and MRHA is not as simple as has previously been assumed. The reason for this discrepancy is unclear.

Finally, the genetic analysis of the K88-mediated adhesion system presents the possibility of manipulating the *adh* cistrons to produce more efficient K88 vaccines. Strains harbouring pMK005 express high levels of K88 on the cell surface, and thus would be ideal candidates for oral vaccine studies. It should be possible to construct non-polar *adhA*⁻ mutants that secrete high levels of the K88 antigen into the culture supernatant and thus facilitate purification of the K88 antigen.

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Molecular cloning and characterization of cDNA sequences coding for rat relaxin

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Using a synthetic oligonucleotide primer, cloned DNA fragments, each containing the entire coding sequence of rat relaxin, have been isolated from a clone 'bank' of ovarian mRNA sequences. The nucleotide sequence of these clones demonstrates that relaxin is synthesized as a preprorelaxin molecule with an unexpectedly large connecting peptide of 105 amino acid residues.

THE peptide hormone relaxin is produced by the corpora lutea of ovaries during pregnancy in many mammalian species, including pigs¹, rats² and humans³, but also surprisingly in a number of non-mammalian groups (notably the shark)⁴. The secretion of the hormone into the blood stream just before parturition results in a marked softening and lengthening of the pubic symphysis and a softening of the cervix which facilitates the birth process. Relaxin also inhibits uterine contractions and may influence the timing of parturition⁵.

The primary structure of porcine relaxin^{4,6,7} consists of two peptide chains of 22 and 31 residues, covalently linked by one intra- and two inter-chain disulphide bonds. The locations of the disulphide bonds are homologous to the structures of insulin and insulin-like growth factors (IGFs)⁸, for which reason it was anticipated that relaxin is also synthesized as a single-chain precursor polypeptide with a connecting peptide linking the A and B chains. Furthermore, by analogy with the structure of proinsulin⁹, it has been suggested that the B chain is at the NH₂-terminus of the precursor molecule.

Nevertheless, porcine relaxin appears to have a precursor of molecular weight (MW) at least 22,000 (22 K) as determined by *in vitro* translation¹⁰, which is much larger than the 11K precursor of insulin, implying either that the connecting peptide of relaxin is far longer than that of insulin (about 30 amino acids) or that there is a peptide extension of the A or B chains which is removed during the processing of the precursor molecule. The recently determined primary structure of rat relaxin¹¹ has, like porcine relaxin, a limited structural homology with insulin, with an identical disposition of half-cystine residues; the A chain has 24 amino acids, the B chain 35 amino acids.

We report here the *in vitro* synthesis and cloning of cDNA transcribed from total mRNA isolated from pregnant rat ovaries into the bacterial plasmid pBR322. Clones containing the coding sequence for relaxin were detected using a relaxin-specific cDNA probe obtained with the aid of a synthetic oligonucleotide primer. Nucleotide sequence analysis elucidated the entire coding sequence for rat relaxin together with the structure of the 3'-untranslated region, and most of the 5'-untranslated region.

Isolation of cDNA clones

The proportions of relaxin-specific mRNA produced by the corpora lutea of ovaries in pregnant rats are not sufficiently high for the convenient detection and isolation of hormone-specific clones (as with insulin mRNA in the islets of Langerhans¹² and growth hormone in the pituitary¹³). Accordingly, we chose first to generate a cloned cDNA 'bank' from total ovarian mRNA and then sought to detect cDNA clones containing the relaxin-coding sequence using an oligonucleotide primer based on the known amino acid sequence of rat relaxin¹¹. (A similar method has been used successfully to detect low levels (0.6%) of gastrin cDNA in a total cDNA population from antral mucosa¹⁴.)

Although the choice of the oligonucleotide primer must be determined from the amino acid sequence of the protein, the degeneracy of the genetic code entails ambiguity at some positions. However, it has been shown¹⁵ that the effects of possible mismatches are minimized by selecting G wherever there is a choice between A and G in the oligonucleotide primer and by selecting T where there is a choice between C and T; the result will either be the correct base pairing or a G-T mismatch. A suitable primer for rat relaxin was chosen by searching through the amino acid sequence¹¹ to find the region (amino acids 6-9 in the B chain, Trp-Met-Asp-Gln, Fig. 1) for which the nucleotide sequence of the mRNA had the minimum number of ambiguities. We obtained the synthetic primer 5'-TGGTCCATCCA-3' containing only one possible mismatch within the first 11 nucleotides of this portion of the coding sequence. This primer, being complementary to the mRNA, was used to generate relaxin-specific cDNA from the priming site on the mRNA template (Fig. 2).

mRNA was prepared from 40 ovaries of rats on days 18–20 of pregnancy (term is 21 days) when relaxin levels are highest¹⁶. Single-stranded cDNA was synthesized using oligo(dT) primer and reverse transcriptase¹⁷ and, after removal of RNA by alkali digestion, converted into double-stranded cDNA by polymerase I (Klenow fragment). This generates a molecule with a hairpin structure on one end and a poly(dA-dT) tract at the other¹². The hairpin structure was removed by S₁ nuclease and poly(dC) homopolymeric extensions were added onto the 3' ends with terminal transferase¹⁸.

A clone bank was obtained by inserting the total cDNA population into the *Pst*I endonuclease cleavage site of the bacterial plasmid pBR322¹⁹, using specific hybridization between poly(dG) tails synthesized on the plasmid and poly(dC) tails on the cDNA. An *Escherichia coli* K12 strain (RR1) was transformed with the recombinant molecules (efficiency = 10^5 clones per μ g plasmid) and colonies were selected by resistance to tetracycline. Individual colonies were screened on nitrocellulose filters²⁰ with a radioactive cDNA probe synthesized from the relaxin-specific primer as described above (Fig. 2).

From a total of ~5,000 recombinant clones, 20 colonies hybridized with the cDNA probe (Fig. 2, lane *e*) and also hybridized positively when rescreened with the specific 150-

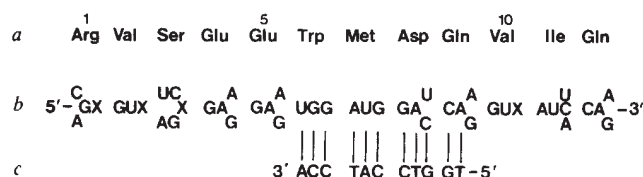


Fig. 1 The choice of an oligonucleotide primer which would specifically hybridize to rat relaxin mRNA. *a*, Amino acids 1-12 of the B chain of rat relaxin. *b*, The hypothetical mRNA sequence coding for rat relaxin, showing ambiguities in the codon choices, where X represents any nucleotide. *c*, The synthetic undecamer finally chosen to be most specific for rat relaxin (see text).

nucleotide fragment eluted from an 8% polyacrylamide gel. Plasmid DNA isolated from 10-ml cultures of each colony was found to contain cloned DNA fragments of 700–900 base pairs. This was not unexpected, because the use of what was essentially a 5'-specific relaxin probe results in the selection of clones carrying nearly full-length cDNA copies of relaxin mRNA.

Extraction of the cDNA insert by cleavage of recombinant plasmids at the *Pst*I sites revealed two types of clone. Sixteen recombinants (type I) contained an internal *Pst*I site in the cDNA insert, giving rise to a 600-base pair DNA fragment (subsequently shown to represent the 3' portion of the mRNA) and a 150–300-base pair DNA fragment (from the 5' region of the mRNA). The remaining four recombinants (type II)

contained inserts of a similar size but without an internal *Pst*I site.

Primary structure of mRNA

The largest plasmid of each type was isolated from 2 l of cells yielding about 1 mg of DNA (pR-RLX1 from type I, pR-RLX2 from type II). The cDNA insert was recovered after *Pst*I digestion and subjected to nucleotide sequence analysis using both chemical and chain termination methods (Fig. 3). The primary structure of relaxin mRNA (Fig. 4) was deduced from the nucleotide sequence of the 840-base pair insert in pR-RLX1. The other type of insert (pR-RLX2) was also completely sequenced, and differed only in the deletion of one nucleotide triplet over the *Pst*I site (GCA or CAG deleted from positions 254–256 or 255–257). The insert within pR-RLX2 was prematurely terminated at nucleotide 77, but was otherwise identical to the structure shown in Fig. 4, thus confirming most of the coding sequence.

The sequence of the 5'-untranslated region does not represent the entire mRNA because the hairpin loop structure of the double-stranded cDNA was removed. However, the size of the specific cDNA synthesized with the relaxin primer (150 nucleotides) suggests that only about 14 nucleotides of the 5'-untranslated sequence are missing from the cloned sequence shown in Fig. 4. It was therefore not surprising to find the clones differing mainly in the size of the 5' end. A repeat of nucleotides 5–18 over 18–31 may be significant in terms of a regulatory function, but sequences in this region of cloned cDNAs must be viewed cautiously due to the possibility of errors²¹ unless confirmed by similar examination of other clones or by direct sequencing of cDNA transcribed from the mRNA¹⁴.

The sequence AAUAAUA in the 3'-untranslated region starting at position 776, about 20 nucleotides from the poly(A) tract, is similar to sequences found in other eukaryotic mRNAs^{22,23} and is probably involved in the addition of the 3'-poly(A) sequences after transcription.

The coding sequence of 560 nucleotides (44–604) contains a nonrandom distribution of codon choices, the significance of which is unknown²⁴.

Amino acid sequence

Figure 4 gives the amino acid sequence for the physiological precursor to rat relaxin (preprorelaxin). The regions corresponding to the known sequences of the A and B chains of rat relaxins are readily identified and the independently derived DNA and peptide sequences for these regions are in complete agreement. The signal peptide of 22 amino acids is followed in order by the B chain (35 residues), a connecting peptide region (105 residues) and the A chain (24 residues). A single stop codon (UGA) follows the cysteine codon for the C-terminal residue of the A chain.

The signal peptide of preprorelaxin contains 22 predominantly hydrophobic amino acids, starting at the initiator codon (AUG, Met) and terminating in Ala before the B chain. There is little homology with the signal peptides of other secreted hormones, apart from the typically high leucine content, which produces some random alignments. A cleavage, presumably by a membrane peptidase after a residue with a small side chain (Ala at position -1), is common of the processing in many signal peptides²⁵. The resultant precursor secreted into the endoplasmic reticulum and eventually being packaged into vesicles at the Golgi apparatus is the 164-residue prorelaxin.

The size of the connecting (C) peptide of 105 residues is one of the surprising features of the prorelaxin structure. One of its functions is presumably to direct folding of the precursor so that the correct disulphide bonds are formed between the B and A chains. However, a connecting bridge of about 30 residues is sufficient to perform this function in proinsulin and even smaller bridges of 11 and 8 residues are found in IGF I and IGF II, respectively²⁶ (Fig. 5). Experiments involving artificial cross-

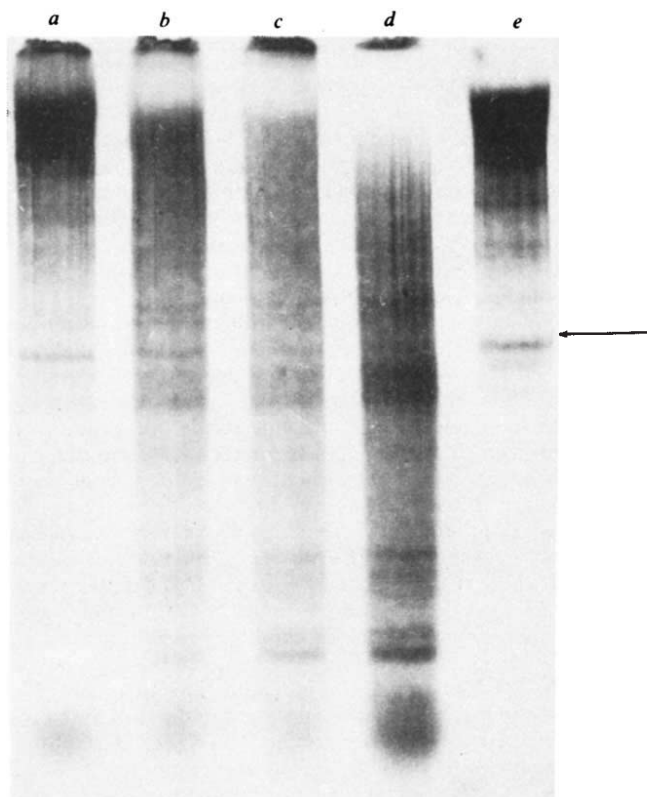
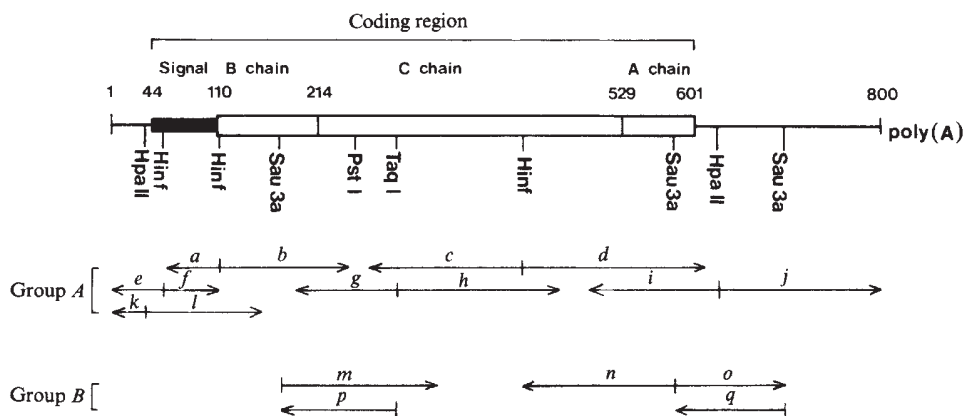


Fig. 2 Primed cDNA synthesis using rat ovarian mRNA and the synthetic undecamer 5'-TGGTCCATCCA-3' (Collaborative Research Products). mRNA was prepared from rat ovaries, taken on days 18–20 of pregnancy, by extraction in 6 M guanidinium thiocyanate¹⁶ and isolation of polyadenylated RNA on oligo(dT) cellulose¹⁷. Aliquots (200 ng) of mRNA were transcribed into cDNA in 10- μ l reactions containing synthetic primer (1 ng), 50 mM Tris-HCl pH 8.0, 50 mM KCl, 7 mM MgCl₂, 1 mM 2-mercaptoethanol, 400 μ M each of dGTP, dCTP, dTTP, 50 nM [α -³²P]dATP (2,000 Ci mmol⁻¹), reverse transcriptase (7 units) and various concentrations of nonradioactive dATP (a, 1 μ M; b, 200 nM; c, 50 nM; d, 0; e, 1 μ M (preparative scale)) at 37 °C for 20 min. cDNA was denatured in 10 μ l of 0.2 M NaOH and heated to 90 °C for 2 min before analysis on a 20 cm $\times$ 20 cm 8% polyacrylamide slab gel (150 V, 3 h). The autoradiograph of the dried gel is shown above (Dupont Cronex MRF-31). The predominant cDNA fragment of ~150 nucleotides length (arrowed) is absent in a primed synthesis of non-pregnant rat ovarian mRNA (data not shown) and therefore is probably transcribed from a pregnancy-specific mRNA expected to be rat relaxin. For use in colony hybridizations 10 μ g of mRNA were transcribed with 5 ng of primer and a dATP concentration of 1 μ M designed to give maximal specific activity in the predominant 150-base cDNA fragment (e, above). The reaction mixture was extracted with phenol-chloroform (1:1 v/v) in the presence of EDTA (10 mM). Unincorporated nucleotide triphosphates were removed in a column (1 cm $\times$ 5 cm) of Sephadex G-50 equilibrated with 0.15 M NaCl, 50 mM Tris-HCl pH 7.5 and 1 mM EDTA. cDNA was precipitated with EtOH; dissolved in 0.1 M NaOH and heated to 70 °C for 20 min to remove mRNA. The total cDNA preparation was neutralized and diluted in Denhardt's solution⁴⁷ before colony hybridization screens on nitrocellulose filters. Alternatively, the cDNA mixture was fractionated on a 8% polyacrylamide slab gel, the predominant 150-base fragment excised from the gel, electroeluted and precipitated for specific colony screens.

Fig. 3 Schematic diagram of the structural gene for rat relaxin. The nucleotide sequence of 800 base pairs was deduced from the largest recombinant plasmid isolated from the colony screen (pR-RX1, insert of about 900 base pairs into pBR322). Nucleotide residues are numbered in the direction 5' to 3' in the message strand, beginning with the first base in the 5'-untranslated region. The poly(dG):poly(dC) homopolymeric extensions of about 20 nucleotides on each end of the insert are not depicted. The coding region for rat preprorelaxin has been divided into the 66-base coding region corresponding to the signal peptide, the 105-base B chain, 315-base connecting or C peptide and 72-base A chain. The poly(A) extension corresponding to the 3'-terminal sequence of the mRNA structure was found to be about 40 nucleotides long. The insert contained an internal *Pst*I recognition site. Thus, cleavage of plasmid pR-RX1 with *Pst*I generated a 300-base fragment (containing the 5'-untranslated region) and a 600-base fragment (containing the 3'-untranslated region and poly(A) tract.) The nucleotide sequence of the cloned DNA plasmid was determined using both the method of Maxam and Gilbert²⁹ and that of Sanger *et al.*³⁰. The plasmid was digested with a selection of restriction enzymes which each form 5'-overhanging ends (*Taq*I, *Hinf*I, *Hpa*II or *Sau*3a as shown). DNA fragments were end-labelled for the Maxam and Gilbert procedure (group A) by incorporating appropriate radionucleotides with reverse transcriptase. Reaction conditions were 50 mM Tris-HCl pH 8.0, 50 mM KCl, 7 mM MgCl₂, 1 mM 2-mercaptoethanol, [α -³²P]dNTP (50 μ Ci, 2,000 Ci mmol⁻¹) and reverse transcriptase (2 units) in a 20- μ l mixture containing the DNA fragment (about 1 μ g) at 37 °C for 20 min. After strand separation in alkali, or digestion with a different enzyme, the unique end-labelled fragments were separated on a polyacrylamide slab gel, then detected by autoradiography or by ethidium staining, and electroeluted. The nucleotide sequence was determined by chemical degradation²⁹ followed by separation of fragments into a sequence ladder on thin 40 cm $\times$ 40 cm polyacrylamide slab gels⁴⁸. Fragments sequenced by this procedure are as follows (the letters in italics refer to the horizontal arrows and the labelled ends of the restriction fragments are designated by an asterisk). *a*, *Hinf*-*Hinf**; *b*, **Hinf*-*Pst*I; *c*, *Pst*I-*Hinf**; *d*, **Hinf*-*Hpa*II; *e*, *Pst*I-*Hinf**; *f*, **Hinf*-*Hinf*; *g*, *Sau*3a-*Taq*I*; *h*, **Taq*I-*Sau*3a; *i*, *Hinf*-*Hpa*II*; *j*, **Hpa*II-*Pst*I; *k*, *Pst*I-*Hpa*II*; *l*, **Hpa*II-*Pst*I. Alternatively (group B), fragments generated by digestion with *Sau*3a or *Taq*I were subcloned (by ligation to the corresponding complementary ends) into the *Bam*HI or *Acc*I site of bacteriophage M13mp7 and grown after transformation of permissive hosts (*E. coli* strain JMB101). The sequence of these subcloned fragments (*m-q*) was determined by primed DNA synthesis using a synthetic primer complementary to the codons for amino acids 13-17 in the β -galactosidase gene. Dideoxynucleotides (P-L Biochemicals) were incorporated using polymerase I (Klenow fragment) in conditions described by Sanger *et al.*³⁰.



links between insulin A and B chains indicate that a bridge equivalent in length to only one or two amino acids is adequate to direct the correct refolding of previously reduced chains²⁷. Thus, the C peptide of prorelaxin vastly exceeds in length the probable minimum requirements for correct alignment of the A and B chains. Although there is no sequence homology between relaxin and insulin C bridges, there is a similar region:

Glu-Asp-Gly-Gly-Pro-Pro-Glu rat relaxin
Glu-Leu-Gly-Gly-Gly-Pro-Glu rat insulin I

with five out of seven identical residues. (Vicinal glycine residues are found in most known insulin C peptides.)

Processing of rat preprorelaxin

As groups of two or more lysine and/or arginine residues are commonly found at sequence regions where processing of peptide hormone precursors occurs²⁵ and are presumed to act as recognition signals for intracellular proteases, it is surprising that there is no cluster of basic residues immediately adjacent to the C-terminal end of the B chain of relaxin. Not all basic clusters, however, are sites for proteolytic cleavage. For example, the rat relaxin A chain contains the sequence Arg-Arg internally and parathyroid hormone contains two internal basic clusters which are not cleaved in the conversion of parathyroid hormone to parathyroid hormone²⁸. In preprorelaxin there is a cluster of four basic amino acids adjacent to the N-terminus of the A chain (Arg-Lys-Lys-Arg). However, the C-terminal residue of the B chain is leucine 35, and it is followed by the sequence

Ser-Gln-Glu-Glu(AGC CAG GAG GAG)

An examination of the nucleotide sequence in this region (Fig. 4) reveals that a deletion of a single base in the codon for the serine would result in the in-phase sequence AGG AGG, which codes for Arg-Arg. Hence, we examined very carefully the possibility of a sequencing error in this region. However, sequencing experiments using both Maxam-Gilbert²⁹ and Sanger³⁰ procedures carried out on both strands of several fragments derived from this region gave unequivocal results in favour of the Ser-Gln-Glu-Glu formulation for the amino acid sequence (see

Fig. 3). Furthermore, it can be seen that such a deletion of a nucleotide in this region would lead to a reading frame in which a stop codon appears within 15 nucleotides at position 237-239. A further possibility we considered was an error in the copying of the mRNA sequence by reverse transcriptase. However, this explanation was ruled out because the identical sequence was obtained for this region from another rat relaxin clone (pR-RX2) (see Fig. 4). This also removes the possibility of a mutation during the cloning procedure itself. A further possibility must be considered, that the processing point is in fact at the next pair of basic residues carboxyl terminal to the present putative cleavage point. This would occur at the Arg-Lys sequence at positions 77-78 of the prorelaxin sequence. In this case, rat relaxin would have a B chain of ~76 residues, and the form which has been isolated from pregnant ovaries and sequenced¹¹ (which has a B chain of 35 residues) would have to be generated from the larger form by further proteolytic cleavage. We believe this is unlikely because the 6,000-MW (6K) form of relaxin was isolated from ovaries frozen at once in liquid nitrogen and stored at -70 °C until workup by a procedure designed to minimize proteolysis³¹. Further, the 6K form of rat relaxin as isolated by two groups^{31,32} is biologically active both in the mouse pubic symphysis bioassay and the rat uterus bioassay. This does not preclude the possibility that there are several forms of rat relaxin generated by variable processing of its precursor. Several high-molecular-weight forms of porcine relaxin have been identified in extracts of pregnant sow ovaries³³. If the precursor to porcine relaxin is similar to rat preprorelaxin in having a large C-peptide bridge, as seems probable from its molecular weight in *in vitro* translation systems¹⁰, these high-molecular-weight forms of immunologically active relaxin could represent the basic 6K form with variable extensions to A and/or B chains resulting from incomplete removal of the C peptide. Such molecules might represent intermediates in the generation of the biologically active relaxin. However, they might also have a different spectrum of biological properties from the 6K relaxin.

Another possible functional role for the large C peptide may be that it contains peptide sequences with other hormonal activities. This would make prorelaxin analogous to the 31K precursor to ACTH and β -endorphin³⁴ in containing more than

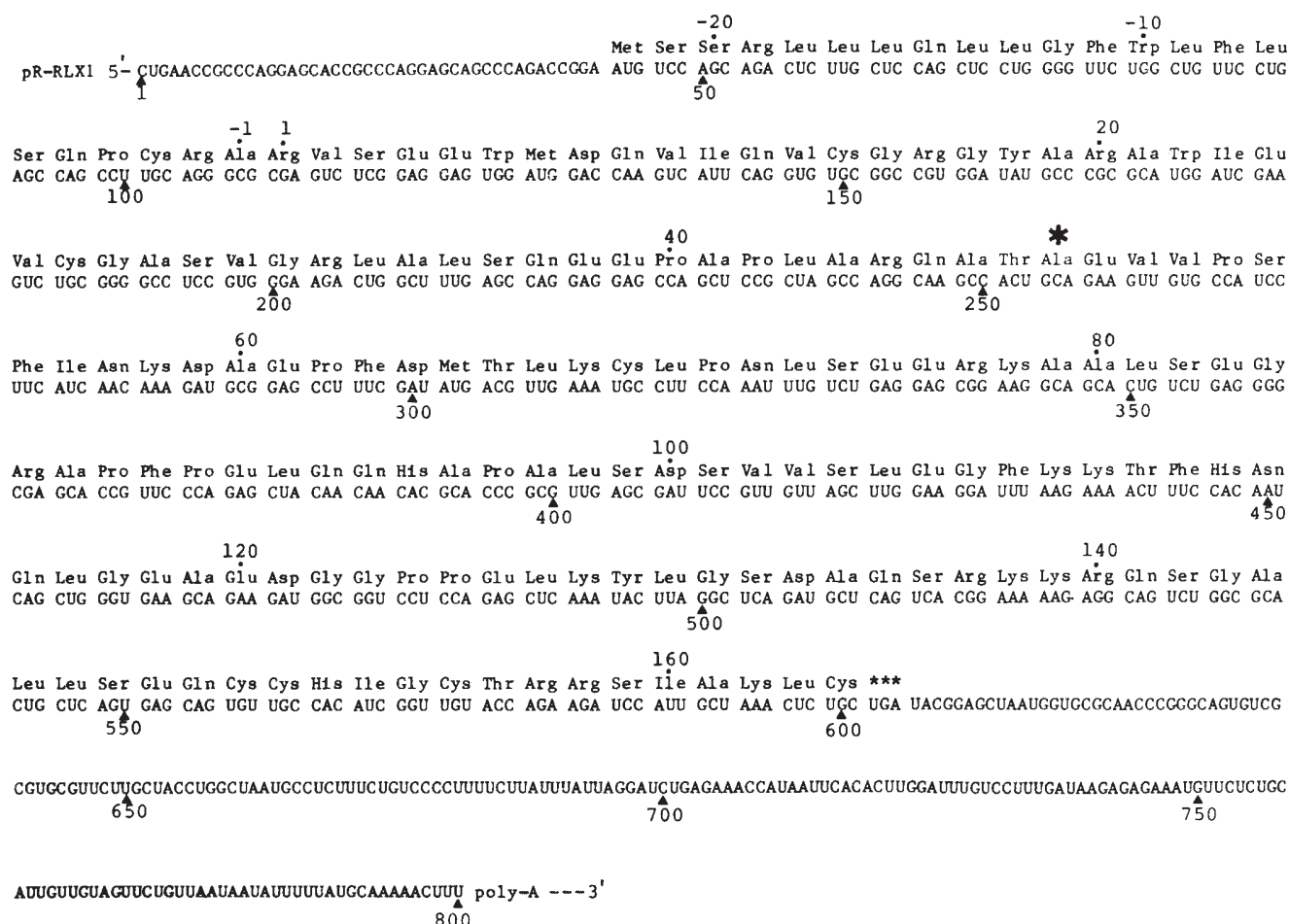


Fig. 4 Primary structure of rat preprorelaxin mRNA. The nucleotide sequence of the mRNA was deduced from that of the 900-base pair cDNA insert in pR-RLX1 (compiled in Fig. 3). Nucleotides are numbered sequentially from the 5' end, being adjacent to the poly(dG) tail in the cDNA. The 5'-terminal sequence is derived solely from one cDNA clone and certainly does not represent the entire sequence of the intact cellular mRNA. However, the 3' end contains a poly(A) tract of about 40 nucleotides and probably represents the exact mRNA structure. The predicted amino acid sequence shown above is numbered sequentially from the amino terminus of the relaxin B-chain sequence through the 164-residue preprorelaxin structure to the termination codon. Therefore, the signal sequence has been numbered from methionine (-22) at the first initiation codon to be found from the 5' end. The nucleotide sequence of a second clone (pR-RLX2) beginning at position 77 was identical over the coding region to pR-RLX1 with the exception of a deletion of alanine 49 (marked by an asterisk). This could represent the deletion of either nucleotides GCA (254-256) or CAG (255-257).

one hormone within its coding sequence. The presence of two groups of dibasic residues at positions 77-78 and 109-110 of preprorelaxin could represent processing points for generation of

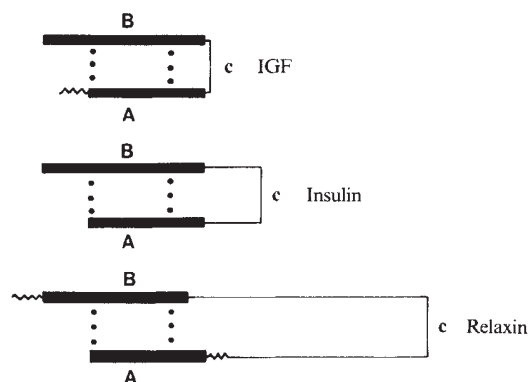


Fig. 5 Schematic comparison of the prohormone structures for insulin-like growth factors⁴⁰, insulin⁴⁰ and relaxin. These structures represent the precursor form of the hormones after the signal peptide has been removed and before subsequent enzymatic cleavage. The precursor molecules all have structures designated from the amino terminus as B chain; connecting (C) peptide; A chain, with the relative size of the C peptides drawn approximately to scale. The wavy lines represent relaxin and IGF A- and B-chain regions which are nonhomologous to insulin.

such putative hormones. At present this suggestion is purely speculative, and based only on the conservation during evolution in at least one and possibly two species of relaxin of what seems to be an unnecessarily large C peptide. There is no information on the fate of the C peptide. However, it should be possible to develop radioimmunoassays using synthetic peptide fragments based on the C-peptide sequence proposed here. Such studies should define whether the C peptide is stored and secreted along with the 6K form of relaxin.

The genes for rat preprorelaxin

The finding of two clones encoding slightly different preprorelaxin molecules was fortuitous and resulted from the fact that the single difference (deletion of a base triplet) removed a *Pst*I cleavage site. It seems unlikely that the difference occurred during cDNA synthesis or cloning, as several examples of each type of clone were found. It is more likely that they represent different preprorelaxin mRNAs, whose translation products would differ only by the presence or absence of alanine at position 49 (within the C-peptide region).

There are several possible explanations for the finding of two preprorelaxin mRNAs. As the mRNA was extracted from pooled ovaries from at least 20 pregnant rats from standard laboratory strains not specially selected for genetic uniformity, allelic variation may account for the two mRNAs. Alternatively

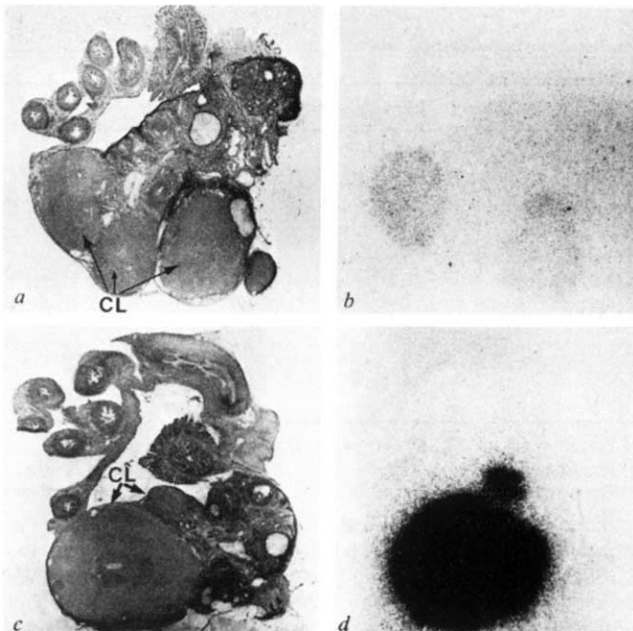


Fig. 6 Hybridization histochemistry⁴³ used to detect the site of hormone synthesis at the mRNA level in rat ovaries. A relaxin-specific radiolabelled cDNA probe was synthesized from 200 ng of the *Hpa*II fragment of pR-RLX1 (nucleotides 40–628). This represents the entire coding region of rat preprorelaxin with only a small portion of the untranslated regions. The synthesis of the radioactive probe followed the procedure of Taylor *et al.*⁴⁹ incorporating [α -³²P]dATP with polymerase I (Klenow fragment) and random primers. Tissue sections (6 μ m) were cut on a cryostat, fixed and hybridized according to a previous method published from this laboratory⁴³. *a*, A non-pregnant rat ovary with corpora lutea marked CL (arrowed) and adjacent stroma, follicles and uterine tube; *b*, autoradiograph of *a* showing background labelling; *c*, a late pregnant rat ovary (18th day) with corpora lutea, adjacent follicles and uterine tube; *d*, autoradiograph of *c* showing heavy labelling of corpora lutea as the site of highest mRNA concentration in the ovary. $\times 10$.

there may be two non-allelic genes for relaxin, as there are for rat insulin³⁵. It may be relevant that the deletion found in type II clones is almost exactly in the position of the intervening sequence within the C-peptide regions of human and chicken insulins^{36,37} and rat insulin II. This raises a further possibility, that the deletion is the result of a minor variation in excision of an intervening sequence. Such a mechanism has been suggested as a possible source of the 20K version of human growth hormone which differs from the usual 22K form by the deletion of residues 32–46 (refs 38, 39).

Whatever the origin of the two relaxin mRNA species, it seems plausible to suggest that an intron may interrupt the

coding sequence for the relaxin C peptide in the genomic DNA. It has previously been proposed that the intervening sequence in the insulin C peptide may account for its being the most highly variable part of the proinsulin molecule, with additions and deletions as well as sequence changes⁴⁰. The striking variability in the size of the C peptide between insulin, relaxin and IGF (Fig. 5) could reflect variations in the relative extent of removal of a common 'genomic C bridge' by successive processing of pre-mRNA and prohormone molecules. These hypotheses can be tested by examining relaxin-encoding clones obtained from cDNA and genomic DNA from individual rats.

Site of hormone biosynthesis in rat ovaries

Immunohistochemical studies have detected relaxin in the corpora lutea of rat ovaries during pregnancy⁴¹ and in cytoplasmic vesicles in pig corpora lutea⁴². These studies support the belief that relaxin is synthesized on ribosomes of the rough endoplasmic reticulum and stored in vesicles of the granulosa lutein cells. We have used an alternative approach, termed hybridization histochemistry⁴³, which has the advantage of being able to detect in specially prepared whole sections of tissue those areas which contain specific mRNA populations. Using a ³²P-labelled cloned cDNA probe corresponding to the coding region of rat preprorelaxin we have identified the major site of mRNA synthesis in ovaries of pregnant rats (Fig. 6). The specificity for the corpora lutea compared with background labelling on adjacent areas of tissue indicates the presence of high levels of relaxin mRNA within the corpora lutea. There was a low level of labelling of the corpora lutea of ovaries from non-pregnant rats due to either nonspecific adsorption or the presence of small amounts of relaxin-specific mRNA in the non-pregnant state. It should be possible to demonstrate the period during which the relaxin gene(s) are expressed by studying the hybridization of the probe to tissue sections taken at various stages throughout pregnancy. Furthermore, when probes corresponding to relaxin mRNA are available from other species such as the human and guinea pig, the technique could be used to examine questions which have been raised^{44,45} concerning the site of relaxin synthesis in tissues other than the ovaries.

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LETTERS

Optical spectrum of the unusual supernova remnant G109.1-1.0

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The new supernova remnant G109.1-1.0 has important X-ray and radio parallels to the SS433-W50 pair. We have obtained a spectrum of the faint optical filaments associated with the large shell-like radio source. The spectrum shows strong [S II] $\lambda\lambda$ 6,717, 6,731 emission relative to H α , which is characteristic of shock-heated gas and which confirms that the filaments are part of the supernova remnant, and is generally similar to spectra of the Cygnus Loop. By assuming pressure equilibrium between the optical filaments and the interior of the remnant, we find an initial energy of 3×10^{51} erg, which is higher than that found from X-ray measurements. When compared with W50, the G109.1-1.0 remnant has filaments of higher density, and weaker [N II] emission.

G109.1-1.0 is a newly discovered galactic supernova remnant (SNR) which has been found to be a source of both X-ray¹ and non thermal radio radiation². The X-ray image obtained with the Einstein Observatory shows an incomplete shell of emission with a diameter of ~ 36 arc min. A strong, compact X-ray source with no known optical counterpart appears at the centre of the X-ray shell. The radio observations show a smaller but complete shell with a spectral index $\alpha \approx -0.5$ ($S_\nu \propto \nu^\alpha$), which is typical for SNRs. These observations also show enhancements in the radio shell along a NE-SW line which approximately line up with a jet-like feature seen both in radio and X ray. This morphology has strong parallels with the association³⁻⁵ between the SNR W50 and the bizarre object SS433.

The distance of G109.1-1.0 has been estimated from its radio surface brightness, Σ , assuming a relationship between Σ and the diameter (D). However, because the angular size of the SNR is different in X-ray and radio maps, and because of differences in the various Σ - D relations, the distance is only roughly known, with estimates ranging from 3.6 to 5.2 kpc. Using the X-ray angular diameter of ~ 36 arc min, and parameterizing to distance $d = 4$ kpc, we find the diameter of the SNR is

$$D = 41.9 \left(\frac{d}{4 \text{ kpc}} \right) \text{ pc}$$

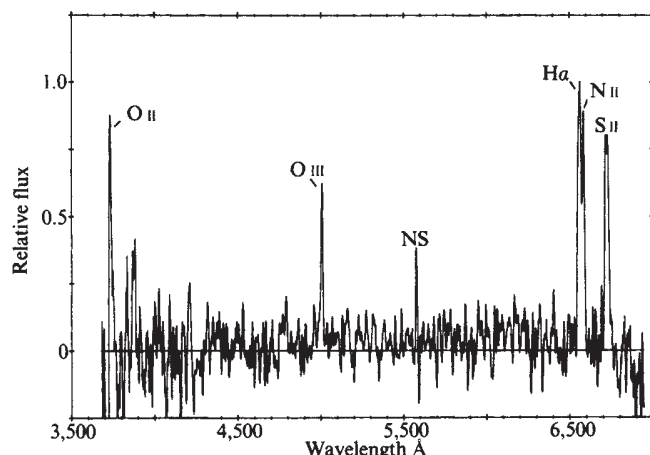


Fig. 1 The optical spectrum of G109.1-1.0 as observed at McGraw-Hill Observatory on 17 October 1980.

Table 1 Line strengths for G109.1-1.0

Line	λ	$F(\text{H}\alpha = 100)$	$I(\text{H}\alpha = 100)^\dagger$	Cygnus‡ Loop-Pos 2
[O II]	3,727	85	762	430
H β	4,861	< 15	< 48	34
[O III]	4,959	15	44	28
[O III]	5,007	52	151	85
[N II]	6,548	29*	29	29
H α	6,563	100	100	100
[N II]	6,584	89	86	87
[S II]	6,717	73	66	72
[S II]	6,731	73	66	55

* Assumes [N II] λ 6,584/ λ 6,548 = 3.0.

† Assumes $E(B-V) = 1.1$.

‡ From ref. 8.

Near the regions of enhanced radio emission are several faint optical filaments visible on the Palomar Observatory Sky Survey (POSS) red plate and on deep H α and [S II] Schmidt plates². We obtained a spectrum of one of these filaments on 17 October 1980 with the intensified Reticon scanner on the 1.3-m telescope at McGraw-Hill Observatory. This filament is located in the southern portion of the remnant at (1950) $\alpha = 22$ h 59 min 45 s, $\delta = 58^\circ 25' 05''$; it runs in a nearly radial direction and is about 2 arc min in length. A 4×40 arc s slit was placed along the filament and alternate 10-min integrations were obtained on the object and on a sky position ~ 10 arc min to the south—well outside the observed emission region. This technique along with the extreme faintness of the filament caused inexact sky subtraction in the resulting spectrum, which is shown reduced to fluxes by standard methods⁶ (Fig. 1). The total integration time on the object was 3,000 s.

The observed line fluxes, $F(\lambda)$, relative to $F(\text{H}\alpha) = 100$ are shown in Table 1. We were not able to detect H β in our spectrum and hence cannot determine the reddening directly. However, the reddening has been estimated for two Sharpless HII regions⁷ nearby which are thought to be at approximately the same distance as the remnant¹. Using a median value of $E(B-V) = 1.1$, we show these reddening corrected line intensities, $I(\lambda)$, in Table 1. The relative line strengths are accurate to 30% except for [O II] λ 3,727, which is worse because the detector sensitivity is poor and rapidly changing in this region.

We have also listed in Table 1 the relative line strengths⁸ of Miller's position 2 in the Cygnus Loop SNR. Comparing these with our observations of G109.1-1.0, we see broad similarities in the relative line strengths. The main difference is that the lines of [O II] λ 3,727 and [O III] $\lambda\lambda$ 4,959, 5,007 are stronger in G109.1-1.0 although the estimates of these lines are particularly vulnerable to the uncertain reddening. The strong lines of [S II] $\lambda\lambda$ 6,717, 6,731 and [N II] λ 6,584 relative to H α are the usual indicators of shock heating⁹ and this confirms that we are seeing the optical filaments of a SNR.

Our spectrum is not good enough to warrant a detailed analysis and comparison with published shock models, but we can use the relative strengths of the [S II] $\lambda\lambda$ 6,717, 6,731 lines to estimate the electron density in the S⁺ zone of the emitting region. Using updated collisional cross-sections for [S II]¹⁰ and a five-level atom calculation, we estimate $N_e(\text{S II}) \approx 700 \text{ cm}^{-2}$.

As the remnant is not bright optically, it is probably still in the adiabatic expansion phase. The optical filaments are probably dense clouds which have been overtaken by the blast wave from the supernova as it travels through the interstellar gas. Using the formulation of McKee and Cowie¹¹, we can estimate the initial energy of the supernova by assuming rough pressure equilibrium between the clouds and intercloud gas (now emitting X rays), given by

$$E_0 = 5.6 \times 10^{43} (\beta')^{-1} N_e(\text{S II}) D(\text{pc})^3 \text{ erg}$$

here β' is a factor of the order of unity which relates the pressure in the cloud and intercloud gas. Using values of $N_e(\text{S II})$ and D mentioned earlier, we find

$$E_0 = 2.88 \times 10^{51} \left(\frac{d}{4 \text{ kpc}} \right)^3 \text{ erg}$$

This is considerably greater than the value of $\sim 7 \times 10^{50}$ erg which has been found for the Cygnus Loop (which is about the same diameter) using the same method⁶. More importantly, it is more than an order of magnitude greater than the initial energy calculated from the X-ray luminosity. Using the X-ray parameters derived by Gregory and Fahlman¹, we find

$$E_0(\text{X ray}) = 1.84 \times 10^{50} \left(\frac{d}{4 \text{ kpc}} \right)^3 \text{ erg}$$

This type of discrepancy for SNR has been noticed previously¹² and may indicate either that the magnetic pressure in the filaments is non-negligible or that there are beams from the central object, as in W50.

The presence of a central radio and X-ray source in G109.1–1.0 and a possible jet-like structure¹ indicate a striking similarity to W50. The emission-line object SS433, which is believed to be associated with W50, also shows a double jet structure in both X rays³ and radio¹³. The axis of this jet seems to be aligned with the enhanced 'radio ear' structure of W50 and it is in this region where faint optical filaments are visible on the POSS red print. Spectra of some of these filaments^{5,14,15} indicate a qualitative similarity to the position we have observed in G109.1–1.0, except that in W50 the [N II] and [S II] lines are much stronger relative to $H\alpha$ and the density as measured from the [S II] lines is lower in W50.

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Cosmological consequences of grand unified theories on density fluctuations

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Recent investigations into the cosmological consequences of grand unified theories (GUTs) of elementary particles have shown that the observed matter–antimatter asymmetry of the Universe can be explained without recourse to the hypothesis of specific initial conditions. It is shown here that the origin of inhomogeneities in the matter distribution, which are thought to be responsible for the later formation of galaxies, cannot be explained by a simple addition of density fluctuations to the standard model. The appearance of these fluctuations, after the epoch when baryon number is fixed, is almost purely adiabatic, any departure from adiabaticity falling off in inverse proportion to the mass of the perturbation.

Fry *et al.*¹ have calculated detailed models for the creation of baryon number in the early stages of the Universe. Their results are parametrized by a quantity $K = 2.9 \times 10^{17} \alpha (m_X/\text{GeV})^{-1}$, where $\alpha \approx 10^{-2}$ is the GUT coupling constant, and m_X is the mass of the superheavy X-boson whose decay is responsible for the generation of baryon number. If $K < 1$, the X-bosons freeze out of thermal equilibrium while they still have relativistic energies, and their number density relative to other particles is independent of the freeze-out temperature and of K . If, on the other hand, $K > 1$, the freeze-out temperature is less than m_X , and the number of X-bosons is reduced by a Boltzmann factor. The resulting ratio B of baryon to photon number then decreases with increasing K .

In models of slightly inhomogeneous cosmologies, one conventionally imagines the Universe to consist of a patchwork of pieces, each larger than the horizon at any given time, and each evolving like a separate Friedmann model with a particular value of curvature². In one of the perturbed regions, the expansion rate will be slightly altered by the curvature term, and this will mean that the freeze-out epoch t_f and temperature T_f will differ from the corresponding values in the unperturbed region (which is taken to have zero curvature). If $K < 1$, this variation in T_f will have no effect on the final value of B , but if $K > 1$, B depends on T_f through the Boltzmann factor. (Strictly speaking, the dependence of B on T_f only vanishes in the limit $T_f/m_X \rightarrow \infty$, but it falls off rapidly as the X-bosons become relativistic at freeze-out.) Consider a perturbed region just entering the horizon at t_f . If the density variation is $\delta\rho/\rho = \kappa$, the expansion rate² will be

$$(\dot{R}/R) = \left(\frac{8\pi G}{3} \rho \right)^{1/2} (1 - \kappa)^{1/2} = \left(\frac{8\pi G}{3} \rho \right)^{1/2} \left(1 - \frac{\kappa}{2} \right) \quad (1)$$

Fry *et al.*¹ give a simple analytic expression for B which applies in the limit $K \gg 1$. Using a variable $z = m_X/T$, then $B = \varepsilon/z_f K$, where ε is the baryon number produced in the decay of an $X\bar{X}$ pair; z_f denotes the freeze-out temperature, and is the larger of the two solutions of

$$K z_f^{7/2} e^{-z_f} = 1 - \frac{\kappa}{2} \quad (2)$$

This equation, although like that given by Fry *et al.*, incorporates the modified expansion rate of equation (1). Now, let z_f be the solution of equation (2) in the unperturbed ($\kappa = 0$) region, and let $z'_f = z_f + dz_f$ be the solution for non-zero κ . It is easy to show that $dz_f/z_f = \kappa/(2z_f - 7)$. The bad behaviour at $z_f = 7/2$ is because equation (2) is an approximation which fails for small z ; we therefore require $z_f \gg 7/2$, and take $dz_f/z_f \approx \kappa/2z_f$. With the expression for B , then $\delta B/B = -dz_f/z_f \approx \kappa/2z_f$. The minus sign means that regions of higher density (positive κ) have smaller values of baryon-to-photon ratio, because freeze-out occurs later in the overdense regions, where the expansion rate is reduced. These conclusions were reached qualitatively by Turner and Schramm³ and by Barrow⁴.

It can be concluded that if K is small, density fluctuations do not alter B : that is, they are entirely adiabatic. If $K > 1$, then departures from adiabaticity can be achieved, but the effect decreases with increasing K (which implies increasing z_f). There must therefore be some pair of values of K and z_f (presumably near unity) for which the non-adiabaticity is maximized. The appearance of the fluctuations is, in a sense, the opposite of isothermal, because the variation in baryon density is smaller than that in radiation density. Note that these departures from adiabaticity have no dynamical significance at these early stages of cosmic evolution, because at these times baryon number is simply a quantum number attached to the various particles comprising the radiation gas. Variations in baryon density reflect variations in the locally averaged baryon number at different points. Only at a later stage ($t \approx 10^{-4}$ s), when nucleons become massive (non-relativistic) particles carrying the baryon number, does the distinction between adiabatic and isothermal fluctuations have a dynamical role.

The result derived above applies directly only to those fluctuations entering the horizon at t_i . A region much larger than the horizon at this time will have the same relation between κ , $\delta\rho/\rho$ and $\delta B/B$ at t_i , but its subsequent evolution must be taken into account. The growth law for large perturbations is $\delta\rho/\rho \propto t$. Bardeen⁵ has shown that the time-dependence of perturbed quantities depends on their definition. The analysis here, using different Friedmann models for different regions, corresponds, in Bardeen's terminology, to a description based on co-moving spatial hypersurfaces, for which the quoted growth law applies in a radiation dominated universe. By the time a large perturbation comes within the horizon, at t_h , its density contrast will have been increased by the factor t_h/t_i . Meanwhile, however, the values of B in perturbed and unperturbed regions will have remained constant, because they are quantities purely local to each region, and so $\delta B/B$ will have also remained constant. Thus the departure from adiabaticity will be reduced by a factor t_h/t_i , and as the horizon mass is proportional to t , the non-adiabatic part of the perturbation falls off in inverse proportion to the mass of the inhomogeneity. Because $t_i \approx 10^{-33}$ s, and t_h can be as large as 10^{12} s for a region entering the horizon at recombination, the character of perturbations large enough to be of interest for galaxy formation will be purely adiabatic to a very high degree.

Hogan⁶ has recently shown that, in a hot big bang, cosmological fluctuations induced by a phase transition at a later stage of cosmic history than is considered here are inevitably insufficient in magnitude to account for galaxy formation. In a 'tepid' big bang, such spontaneous fluctuations may be strong enough, but this requires $B \geq 1$ in the early stages⁶, which cannot be provided by GUTs⁷. I have shown here that density fluctua-

tions built into the initial conditions of the Universe are also incapable of being directly responsible for large matter inhomogeneities. A possible solution to this dilemma may lie in the generation of inhomogeneities through some kind of non-linear phenomenon (such as the 'Purcell effect'⁸, although this requires strong adiabatic fluctuations, $\delta\rho/\rho \approx 10\%$), or perhaps through the use of evaporating primordial black holes as a source of baryons³.

Barrow and Turner⁹ have performed a similar calculation to that presented here, but suggest that anisotropic (shear) fluctuations could produce useful isothermal variations because they alter the expansion rate at early times, but, unlike the modes considered here, subsequently decay rather than grow. Although the assumed presence of shear inhomogeneities is reasonable, at the same time one must ensure that density fluctuations are absent, to better than ~ 1 part in 10^{40} of the anisotropic modes, otherwise they will eventually grow and dominate. It thus seems that rather special initial conditions would be needed to create significant isothermal fluctuations.

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Meteoroid impact into short-period comet nuclei

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After their introduction into the inner Solar System, most short-period comets spend the majority of each orbit within the asteroid belt. Provided that cometary material is similar to terrestrial snow in its shock behaviour, the nuclei would accumulate meteoroids as a consequence of collisions with asteroidal debris. Here the effects which extended meteoroid bombardment might have on short-period comet nuclei in ideal conditions are evaluated; the results are extrapolated to more probable situations, and their possible implications for asteroid and meteorite studies are considered. It is suggested that after devolatilization of the nucleus, the accumulated material might combine with primary cometary solids to produce environments conducive to the formation of polymict brecciated meteorites and to influence reflectance spectra of the 'asteroid'. In addition, such meteoroids removed from active nuclei during degassing could be placed in trajectories which might evolve into Earth-crossing orbits.

It is assumed here that comet nuclei are H₂O-rich bodies with weak, probably porous structures, containing a non-negligible fraction of non-volatile 'dust'. Deduction of various physical properties suggests that the impact behaviour of nuclear material can be approximated by that of terrestrial snow (Table 1). Experimental studies reveal that a projectile impacting slightly packed snow will penetrate to many multiples of its own length²; its final location is generally at the apex of a conical 'crater'²; and the 'crater' is characterized by a large

depth/diameter ratio, apparently due to a large amount of target compression³. A least-squares fit to data by Swinow² for the penetration (L) of 0.55-cm metal cubes into snow (0.41 – 0.42 g cm⁻³) yields

$$L = 0.2 d \rho^{1.045} V_i^{0.349} \quad (1)$$

where d , ρ and V_i are the projectile length, density and impact velocity, respectively (all variables in c.g.s. units). While some of the experiments used in deriving equation (1) involved impact velocities lower (≤ 1.3 km s⁻¹) than those considered to be ideal, many were conducted in conditions expected to apply for the cases treated below—within or near the snow-ice transition regime^{4,5}. As spallation will remove mass from silicate projectiles during their penetration at high velocities, equation (1) should yield upper limits to penetration depths.

Assuming that a comet is perturbed into an orbit lying completely within the asteroid belt, then the total mass flux (ϕ) of particles impacting a non-gravitating surface of unit area can be written as

$$\phi = \frac{k\alpha}{\alpha+1} (m_0^{\alpha+1} - M^{\alpha+1}) \quad (2)$$

where k and α are constants, m_0 is the minimum particle mass

Table 1 Brief comparison of the physical properties of H₂O snow and those deduced for comet nuclei

Property	H ₂ O snow Value	Ref.	Comet nuclei Value	Ref.
Density	0.1–0.9 g cm ⁻³	22	–0.2–1.3* g cm ⁻³	7, 23*
Compressive strength	10^4 – 5×10^7 dyn cm ⁻² †	5	10^4 – 10^6 dyn cm ⁻²	24
Tensile strength	10^4 – 10^7 dyn cm ⁻² †	5	$\sim 10^3$ dyn cm ⁻²	25

* Includes nonvolatile solids. † Over a density range of 0.1–0.9 g cm⁻³.

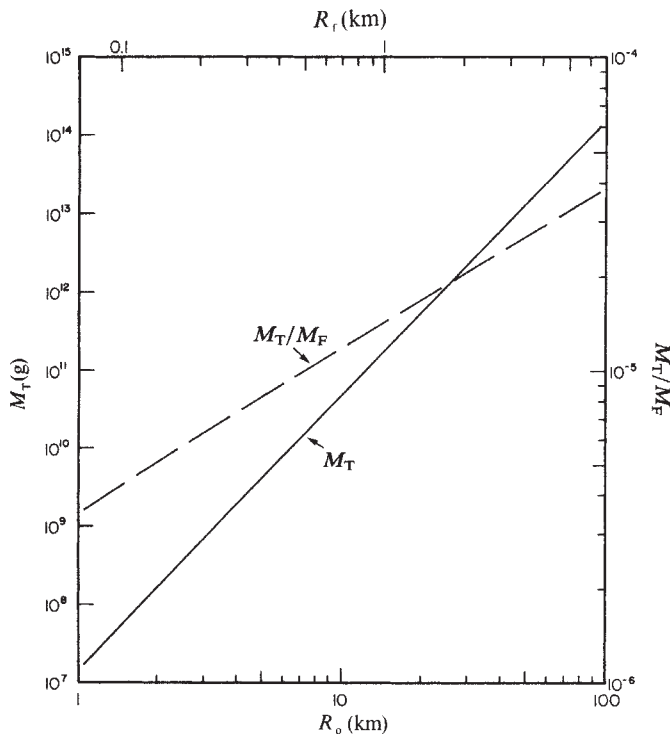


Fig. 1 Total mass of debris (M_T) accumulated by comet nuclei of various initial radii (R_0). M_F is the mass of the burned-out nucleus, exclusive of the accumulated debris, and R_f is the final radius of the devolatilized nucleus. For comparison, the lunar mare basalts comprise between 4×10^{-5} and 4×10^{-4} times the total lunar mass.

that can be retained against gas drag during the nucleus' devolatilization, and M is the largest mass which could be stopped within the average chord length of the (assumed spherical) nucleus. Projectiles with masses greater than M will either disrupt or completely penetrate the nucleus, the former of which would nullify the applicability of the following calculations. Using the equation derived by Whipple¹ to fix m_0 and equation (1) to establish M results in

$$\phi(t) = \frac{k\alpha}{\alpha + 1} \left[\left(\frac{2.87 \times 10^{19} \rho}{nq^{27/4} R_c^3(t)} \right)^{\alpha+1} - (253.67 R_c^3(t) \rho^{-2.135} V_i^{-1.047})^{\alpha+1} \right] \quad (3)$$

where $1/n$ is the fraction of incident solar radiation used in subliming ices and $R_c(t)$ is the time-dependent nuclear radius. The perihelion (q) is expressed in astronomical units. Taking the rate of change of the nuclear radius (λ) to be constant, its surface area at time t can be written as

$$A(t) = 4\pi R_c^2(t) = 4\pi(R_0 - \lambda t)^2 \quad (4)$$

where R_0 is the initial radius. The total mass acquired by the nucleus from time 0 (introduction to the asteroid belt) to time T (total devolatilization) can be found from

$$M_T = \int_0^T \phi(t) A(t) dt \quad (5)$$

Only silicate projectiles will be treated here, making a chondritic density of 3.5 g cm^{-3} appropriate, and the average asteroidal encounter velocity of 5 km s^{-1} (ref. 6) will be used for this case. Conservative values of 2.0 AU and 1 will be assumed for q and n , respectively. Estimates made by Sekanina⁷ yielded a value of $2.84 \times 10^{-6} \text{ cm s}^{-1}$ for λ and a mean final radius (R_f) of $0.069 R_0$. The impact flux in the asteroid belt suggested by Housen *et al.*⁸ will be adopted, setting k and α at 4.74×10^{-17} and -0.833 ,

respectively. Along with the fact that $R_f = R_0 - \lambda T$, inserting these quantities in equation (5) yields

$$M_T = 3.5 \times 10^{-10} (0.139 R_0^{3.501} - 1.204 \times 10^3 R_0^{2.499}) \text{ g} \quad (6)$$

The results of equation (6) for nuclei of various initial radii are shown in Fig. 1 which also includes the total accumulated masses normalized to the final nuclear mass (assuming $R_f = 0.069 R_0$ and a density of 3.0 g cm^{-3}).

It is anticipated that these former projectiles would, after devolatilization of the nucleus, be concentrated in the near-surface layers of the 'regolith'. The surface density of this 'foreign' debris (assuming an even distribution over the surface) can be found by dividing equation (6) by the final surface area of the nucleus ($A_f = 4\pi R_f^2$)

$$\mu = 5.85 \times 10^{-9} (0.139 R_0^{1.501} - 1.204 \times 10^3 R_0^{0.499}) \text{ g cm}^{-2} \quad (7)$$

which, in turn, can be translated into an effective thickness (h) by

$$h(p) = \frac{\mu}{\rho(1-p)} \quad (8)$$

where p is the porosity of the surface layer. Values of $h(p)$ are plotted in Fig. 2 for various porosities and initial nuclear radii.

The orbit used in the calculations is not typical of most short-period comets⁹, as most observed orbits are significantly more eccentric than that assumed here. Comets with highly inclined orbits are excluded from discussion, as they would spend negligible time in the plane of the asteroid belt. The more eccentric orbits will increase the average impact velocity of asteroid debris. Equation (3) shows, however, that the effects on $\phi(t)$ will be minor: a difference in V_i of a factor of ± 5 will change $\phi(t)$ by a factor of only ∓ 0.3 . The time spent outside the belt (hence, in a less frenzied impact environment) will also depend on the orbital eccentricity. Nonetheless, most short-period comets with aphelia inside the orbit of Jupiter spend a large fraction ($\geq 75\%$) of each orbital period within the asteroid belt¹⁰, thus placing minimal restrictions on the model results. Finally, the values of λ and $1/n$ used for the model orbit are almost certainly too high. At an orbital average distance of 2.8 AU, for example, water snow will sublime at $\sim 9.2 \times 10^{-7} \text{ cm s}^{-1}$ (ref. 11)—a rate lower than that used above by more than a factor of 3. Considering the fact that the average perihelion distance of the objects used in deriving λ is $\sim 1.3 \text{ AU}$, the results given by equation (6) should be more appropriate for the

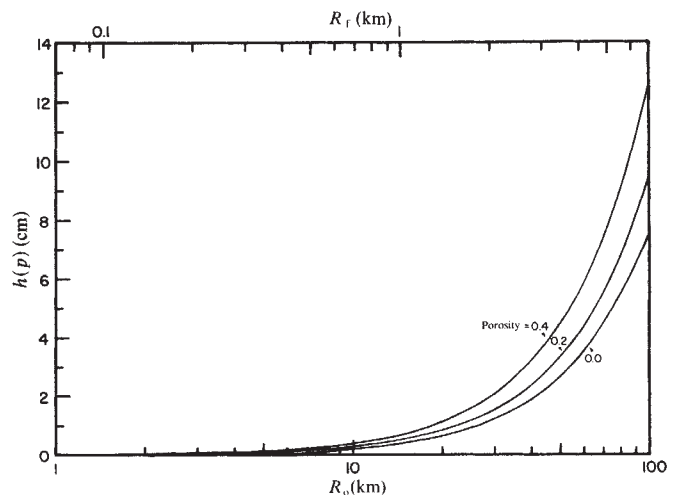


Fig. 2 Effective thickness of the accumulated debris layer as a function of porosity [$h(p)$] compared with the initial and final radii of the nucleus (R_0 and R_f , respectively). Most natural fragmental deposits have porosities of ~ 0.4 . Projectile density: 3.5 g cm^{-3} .

Effective surface layer density (g cm^{-3}):	3.5	2.8	2.1
Porosity:	0	0.2	0.4

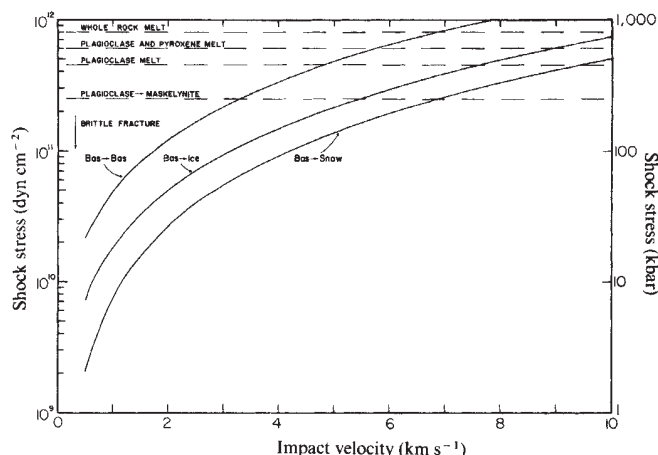


Fig. 3 One-dimensional shock stress as a function of impact velocity for basalt projectiles incident on basalt, H₂O ice, and Greenland snow. Dashed horizontal lines mark the stresses at which the indicated phase changes were observed to occur in experimentally shocked basalts²¹.

average rate of radius decrease for more eccentric orbits. While ejection and vaporization of volatile and non-volatile material during impact events would also contribute to mass loss from the nucleus, its magnitude relative to the solar irradiation-induced component is unknown. The value of 1 used for $1/n$ implies that the albedo of the nucleus is effectively zero, thus yielding the unlikely condition that all incident radiation is absorbed and used in subliming the volatile ices. Clearly, this is not the case; a smaller average fraction of solar radiation will be partitioned into devolatilization of the nucleus. These values for λ and $1/n$ will yield a shorter lifetime for a given nucleus than would actually be the case. The calculated masses and effective thicknesses of debris should therefore be treated as conservative estimates *vis à vis* these parameters.

According to recent evidence¹², polymict brecciated meteorites might have been formed during low-velocity collisions between asteroids of dissimilar compositions. Another mechanism which could also account for some of these meteorite types might be the shock lithification of the variegated regolith of a burned-out short-period comet nucleus, such as that described above. Stresses sufficiently high to indurate particulate regoliths would be generated at the impact velocities expected to occur in the asteroid belt¹⁰. Thus, the generation of polymict breccias on such 'foster-parent' bodies would be directly analogous to the formation of their lunar counterparts. Insofar as the foreign fragments were, in all probability, originally components of asteroid regoliths^{8,13}, the observed abundance of implanted solar wind gases and solar flare/cosmic ray tracks should be consistent with this model.

Such a regolith would present a reflectance spectrum characteristic of the averaged behaviour of its component lithologies and/or mineralogies. While often considered to be carbonaceous, the composition of primary cometary debris is uncertain^{13,14}. Thus, the spectral signature of a devolatilized nucleus cannot be predicted with confidence, surface exposures of foreign debris notwithstanding. For this reason, it would be a formidable task to select candidate objects on the basis of spectrophotometric grounds alone. Note that observations would be biased towards the larger objects, implying that the original (volatile-rich) nuclei would also have been large relative to most presently-active short-period comets¹⁵. This model predicts that more massive nuclei would have collected more debris, thus maximizing any spectral effects resulting from the overall process. 'Mixing models' similar to those applied by Chapman and Gaffey¹⁶ might yield information relating to the efficiency of this mechanism. Orbital and dynamical studies of

fireball and meteorite orbits present strong arguments in favour of some relationships between meteoroids and short-period comets^{17,18}. Objections presented by petrologic and chemical arguments against cometary origins of non-carbonaceous meteorites^{13,19} might be alleviated if the comets acquired the meteoroids during passage through the asteroid belt or at some earlier time in their histories. Figure 3 illustrates that no petrographically observable shock effects other than brittle fracture would occur in basalt-like assemblages as a result of collisions with comet nuclei at velocities lower than $\sim 5\text{--}7\text{ km s}^{-1}$. Comets in highly eccentric orbits (which characterize Earth-crossing comets, such as P/Encke) would encounter asteroidal debris at higher velocities (D. R. Davies and E. M. Shoemaker, personal communications), forcing the projectiles to incur greater shock damage. Fragments 'absorbed' by short-period comets in less elliptical orbits, however, would be subjected to relatively mild stress levels. Ejection of these meteoroids during periods of devolatilization would place them in orbits similar to that of the nucleus. If the nucleus' orbit were conducive to Jupiter-related perturbations¹⁶, or if it had become Earth-crossing (for example, due to perturbations by Jupiter, or by nongravitational forces²⁰), the ejected fragments would then be in orbits which could ultimately encounter the Earth. The total annual mass which might be delivered to the Earth by this mechanism is uncertain, but large fragments (of the order of $10^4\text{--}10^6\text{ g}$, depending on the nuclear radius, perihelion, and so on¹) can be removed from nuclei by gas drag. Because of the ease with which they lose solid debris, small nuclei should be more effective protagonists in this process.

Finally, a spacecraft with high-resolution ($\leq 1\text{ m}$) imaging, multispectral, and/or chemical mapping capability could survey the surface exposure of material on an active short-period comet nucleus. Such an investigation would provide important data regarding the composition, abundance and dispersal of debris in the regions of the asteroid belt traversed by the comet during its active lifetime.

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Coseismic changes in groundwater temperature of the Usu volcanic region

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Observations of groundwater temperature to an accuracy of 0.001°C or better have been made at about 30 wells in various areas of impending earthquakes in Japan. The main aim of the observations was to monitor the crustal strain and/or the movement of groundwater which relate to earthquake occurrence. One thermometer was installed at a depth of 200 m in an active volcanic area, Usu, where an upward movement of the magma is occurring. We report here that there was a generally increasing temperature trend at this location: 0.3°C in the half-year of observation. Decreases in the rate of increase preceded intense local earthquake activity. Temperature jumps were accompanied by large local earthquakes with magnitudes up to 4.2, and the polarity of the steps corresponded to the location of hypocentres. This is probably the first micro-temperature measurement made in the proximity of seismic activity.

Usu volcano, 70 km south-west of Sapporo, erupted on 7 August 1977, after lying dormant for ~ 30 yr. A series of eruptions followed the first one, continuing until October 1978. Many earthquakes have been observed in the area, beginning just before the first eruption and continuing to the present. The maximum magnitude of the earthquakes was 4.3. Although the earthquake activity has been gradually decreasing since the very first stage of Usu's eruption, it remains at a much higher level than that before the eruption. About two to four earthquakes of magnitude 4 are still occurring every month, so that a lot of small earthquakes are being observed by a local seismological network, consisting of 10 observation sites operated by the Usu volcanological observatory of Hokkaido University.

A precision quartz thermometer was placed in an observation well of depth 380 m, 2 km east of Usu volcano in October 1979. The quartz sensor was installed at a depth of 200 m (below this the well was too narrow). Our thermometer is accurate to 0.001°C , has long-term stability, and is specially designed for geophysical field observations¹. Our main aim was to monitor movements of groundwater which were probably related to changes in crustal stress and earthquake generation. Such observation is also interesting from the geothermal viewpoint, as an upward intrusion of magma beneath the volcano has been suggested². Seismologically, this observation is a rare opportunity to observe many earthquakes with magnitude 4 at epicentral distances of less than several kilometres.

In general, the results show a nearly monotonic increase in the groundwater temperature (Fig. 1), which contrasts with obser-

vations in other regions; the increment being $\sim 0.3^{\circ}\text{C}$ since the start of the observation. However the rate of increment has been slightly decreasing, which probably relates to the gradual decrease in volcanic activity such as crustal deformation or energy release. The thermometer has been tested for long-term stability and found to have a drift rate of less than $0.01^{\circ}\text{C yr}^{-1}$, so that the observed increment cannot be due to instrumental error.

About 30 other thermometers have been installed since 1978 in various areas of impending earthquakes in Japan¹. However, the Usu thermometer is the only one to have been deployed in an active volcanic area. Hence the general increase in recorded temperature is likely to be due to the upward movement of the magma that has been shown by various volcanological studies². Other thermometers did not record such a large increment in the groundwater temperature, probably because there was no region in which a very shallow magma was rising. For the other wells the d.c.-shifts of the groundwater temperature are $< 0.03^{\circ}\text{C}$; the present case is more than 10 times larger than those.

Fluctuations in the generally increasing rate are shown in Fig. 1. The decreases in the increasing rate precede high levels of earthquake activity. For example, they are obvious before 13 April, 30 April, and 12 May. Sometimes the ascending trend was even reversed just before intense activity. This applies to periods other than those illustrated in Fig. 1. The intense seismic activities are recurrent, but not periodic; hence we conclude that the 'predictions' of seismic activity by the change in the increasing rate of the observed temperature are caused by some physical mechanism.

It is also interesting that 'seismograms of temperature changes' are frequently recorded by the thermometer (Fig. 2). Almost all large earthquakes were recorded with sharp spikes, and most of them resulted in offsets of the temperature, as is seen in Fig. 2. The onsets of sharp spikes coincide with earthquakes within the accuracy of the sampling interval of the digital thermometer, 15 min; the peaks were reached rapidly, within the same sampling interval of the onset. On the other hand, the average time taken for the spikes to decay was a few tens of minutes. Sometimes the sampling interval was 1 h, as in Fig. 2, instead of 15 min. The amplitudes of the spikes are proportional to the local seismic intensity. The thermometer system was designed to be immune to strong accelerations in the near field of earthquakes and because it was developed to be installed in areas of impending earthquakes, there are no movable parts either in the sensing unit or in the recording unit. It was also tested for exposure to vibrations. We postulate therefore that the recorded spikes were due to frictional heat generated between the pressure case of the sensing unit and the surrounding water in the well as a result of the shaking caused by earthquakes.

Hence the spikes have little geophysical meaning except that we can estimate the thermal time constant with which the generated heat is diffused from the sensing element to the outer groundwater. In contrast, there are substantial offsets remaining

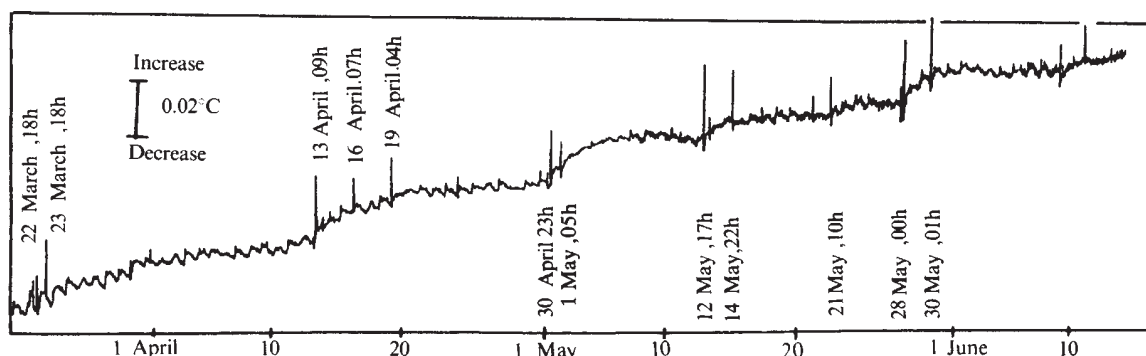


Fig. 1 Record of the groundwater temperature at Usu volcano, obtained by a precision quartz thermometer with an accuracy of 0.001°C . Spikes correspond to large earthquakes, whose dates and times are indicated.

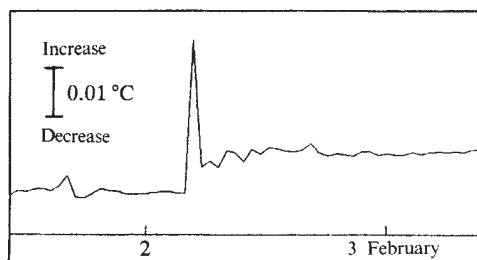


Fig. 2 A 'seismogram' recorded by the quartz thermometer. The record is characterized by a sharp spike which is accompanied by an offset of the groundwater temperature. This record was taken at the sampling interval of 1 h; the leading edges of the spikes actually have steeper onsets. Epicentre, Kita-byobu. Magnitude, 4.1.

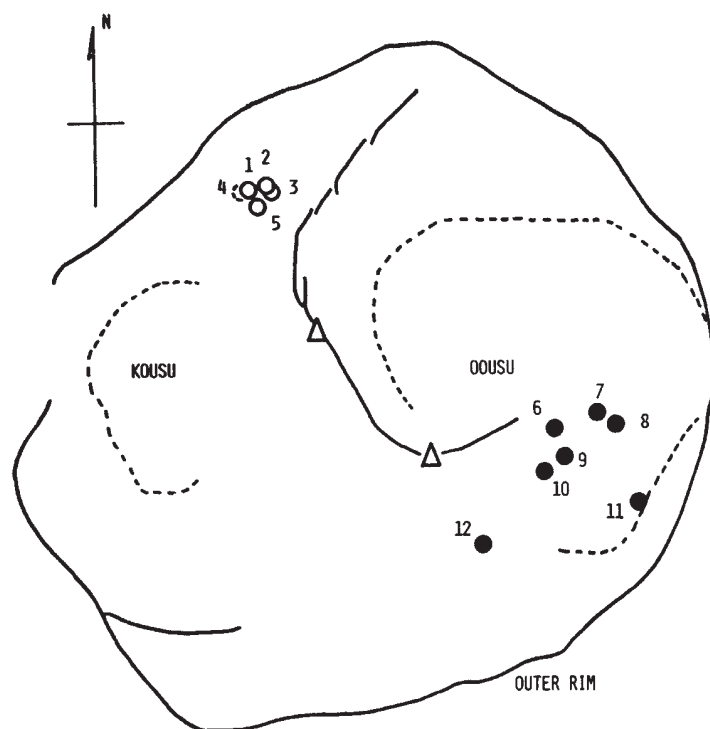


Table 1 Magnitude and dates of earthquakes shown in Fig. 3

No.	Date (1980)	Mag	Temperature step (°C)
1	13 April	4.1	+0.0024
2	2 February	4.1	+0.0062
3	8 March	4.2	+0.01
4	23 March	4.1	+
5	23 January	4.2	+0.0063
6	23 March	3.9	-0.002
7	27 February	4.0	-0.0038
8	12 May	4.1	-0.003
9	30 April	3.9	-0.002
10	22 March	4.0	-0.0046
11	24 April	3.9	-0.001
12	14 May	3.9	-0.001

Fig. 3 The observation area, the earthquake epicentres and the location of the observation well. ●, Earthquakes with negative temperature offsets; ○, earthquakes with positive temperature offsets. The U-shaped solid line in the crater is a major surface fault zone. The earthquakes are listed in Table 1. Temperature offsets without a numeral indicate that the amount was too small to be reliably read, although polarity was positive. For magnitudes and dates of earthquakes see Table 1.

after the spikes: both positive and negative offsets were observed which indicates that the recorded offsets are not instrumentally caused by the strong vibration, because the local accelerations of the earthquakes were identical for the two groups which caused inverse polarities. We found that the polarity of the offsets are definitely determined by the locations of earthquake foci; the amounts of the offsets are closely related to earthquake magnitude (Fig. 3). However, we have not found a relationship between the amplitudes of the offsets and the focal depths that were distributed from the surface to 1.2 km.

The largest positive offset, +0.01 °C, was recorded during a magnitude 4.2 earthquake on 8 March 1980. All the group earthquakes (including the magnitude 4.2 earthquakes) that were associated with the positive offsets, occurred beneath the Kitabyobu region, which is the north-west part of the crater of the volcano. The group of earthquakes associated with negative offsets occurred, also without exception, beneath Oousu-minami, which is the south-east part of the crater. The mean epicentral distances of the two groups from the observation well were 2.7 and 1.5 km respectively.

These groups of earthquakes have their own focal mechanisms, which are almost identical within the groups: reverse fault for Kitabyobu, normal fault for Oousu-minami. Both mechanisms have fault planes which are steeply dipping. The fault mechanisms are consistent with a thrust movement, towards the north-east, of the U-shaped surface fault zone,

which has been observed by geodetic measurements and geological observations.

At Usu volcano, large earthquakes occur only in these two regions. Smaller earthquakes are more diverse, covering almost all the crater region, and some occur beyond the outer rim, a few kilometres north-east. We did not observe offsets of the groundwater temperature for earthquakes whose magnitudes were <3.7, regardless of their focal locations, thus we do not know whether these small earthquakes generate smaller offsets beyond our detection capability, or whether they inherently do not generate offsets.

We do not have a definite physical explanation for the observed phenomena, the steep trend of the increase in groundwater temperature and the offsets of the temperature. However, the former is probably related to the upward movement of the magma, and the latter to a sudden release of the pore pressure when an earthquake occurs. If so, the monitoring of the pore pressure by the precise monitoring of the groundwater temperature is a promising technique for predicting earthquakes.

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Recent glacier variations and volcanic eruptions

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The injection of volcanic dust and gases into the atmosphere during major eruptions has been advanced to explain short-term variations of climate¹⁻⁴. A calculated global cooling of ~1 K during episodes of intense volcanic activity³ could lead to a snowline depression sufficient to cause glacier advances equivalent to those of the last several centuries. The hypothesis would therefore be strengthened if a close relationship could be demonstrated between global volcanicity and the pattern of recent glacier variations. Glacier activity in the Northern Hemisphere during much of the past 100 yr shows a lack of synchrony with that in the Southern Hemisphere, but in each hemisphere the sequence of glacier variations matches the acidity record in polar ice cores and the frequency of volcanic eruptions in successive latitude belts. This suggests that glaciers fluctuate in response to atmospheric build-up of volcanic aerosols produced during large eruptions and that explosive volcanism may therefore be a major factor in modulating climate on the decadal scale.

Temperate mountain glaciers are an important source of proxy climatic data, for they respond rapidly to variations of climate by changing in size. A relationship between glacier fluctuations and volcanic episodes would imply a possible link between eruptive activity and climatic change. Fluctuations of many middle-latitude glaciers have been documented during the present century, and for certain glaciers discontinuous observations extend back into the nineteenth, eighteenth or even seventeenth centuries. For glaciers where such data do not exist or are incomplete, records of marginal variations during the past several hundred years often can be reconstructed and dated using geological and botanical methods⁵.

Active volcanoes, defined as those which have erupted at least once since AD 1800, are concentrated disproportionately in the Northern Hemisphere; most lie between 60°N and 10°S latitude near the margins of converging lithospheric plates⁶ (Fig. 1a). Few are found south of latitude 20°S, and their number drops off sharply in both hemispheres polewards of 60°. This asymmetrical global distribution suggests that cumulative long-term loading of the atmosphere by volcanic aerosols may vary with latitude. This inference is supported by Lamb's plot showing the frequency of large dust-producing eruptions as a function of latitude which is dominated by eruptions in equatorial and northern middle latitudes (Fig. 1b). Dust entering the upper atmosphere at low latitudes is likely to disperse into both hemispheres, whereas dust injected at high latitudes may remain largely in one hemisphere^{1,3}. Consequently, if large volcanic events do affect climate, then differing frequencies of major eruptions in successive latitude belts might produce contrasting climatic responses that could generate asynchronous patterns of glacier behaviour in the two hemispheres.

Most detailed records of glacier variations in the Northern Hemisphere come from mountainous areas lying between 40° and 70°N latitude (especially Scandinavia, the Alps and north-western North America) (Fig. 2); although the mountain ranges of central Asia contain many glaciers, sporadic observations, often separated by several decades⁷, and the presence of surging glaciers make that region unsuitable for detailed analysis. Glacier advances during the past 100 yr culminated mainly between about 1880 and 1895 and between 1910 and 1925. An intermediate advance, climaxing between about 1900 and 1906, has been reported only south of latitude 62°. An episode of

renewed advance began as early as the middle 1950s but increasingly was detected in the 1960s throughout northern middle latitudes. Widespread retreat of glaciers marked the interval between the middle 1920s and the early 1950s, with recession being most pronounced in the 1930s and 1940s⁸⁻¹¹. Although there were a few reported advances during this interval, most involved surging glaciers. This period of marked retreat, one of the most striking features of the recent Northern Hemisphere glacial record, has attracted much attention, for not only did many glaciers experience considerable reduction in size, but some disappeared entirely.

The few mountain areas at low latitudes that support glaciers are poorly studied, but the limited data available suggest a pattern of fluctuations similar to that of northern middle latitudes.

Southern Hemisphere data come mainly from New Zealand and the Andes, although limited information is also available for certain sub-Antarctic islands. There are fewer recorded observations than for the Northern Hemisphere (Fig. 2) and conclusions based on them are, therefore, less reliable. Despite the large number of glaciers in the Andes, the record from South America is less well known than that from New Zealand and represents a smaller sample. Some of the best-documented advances in the southern Andes involve possible surging glaciers and were excluded from consideration. Glacier advances in the Southern Hemisphere culminated between the middle 1870s and the middle 1890s, between 1906 and 1910, in the first half of the 1930s and in the late 1940s. A recent renewed advance began in the late 1950s to early 1960s, slightly later than in the Northern Hemisphere.

In spite of the common view that glacier fluctuations have been in phase globally during the past 100 yr, in fact they were largely asynchronous between the Northern and Southern Hemispheres for much of this period (Fig. 2). Although a widespread re-advance of glaciers characterized the final decades of the nineteenth century in both hemispheres, a subsequent re-advance in northern middle latitudes that peaked between 1910 and 1925 preceded one in southern middle latitudes which culminated in the early to middle 1930s, a time when Northern Hemisphere glaciers were experiencing their most significant period of retreat. An advance of New Zealand

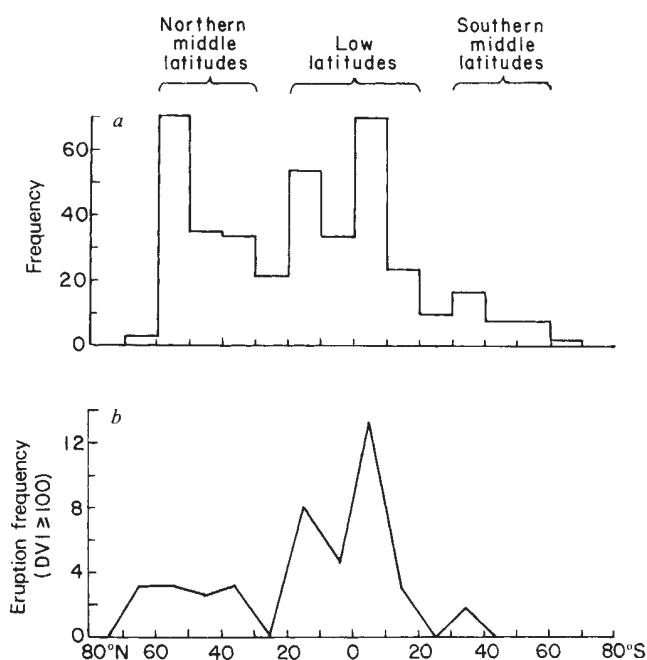


Fig. 1 *a*, Distribution of active volcanoes as a function of latitude. Data compiled from ref. 6. *b*, Number of eruptions per 10° latitude band producing a dust veil index (DVI) of ≥ 100 (excluding area south of 50°S) for the period 1800–1964 (from ref. 1, Fig. 19a).

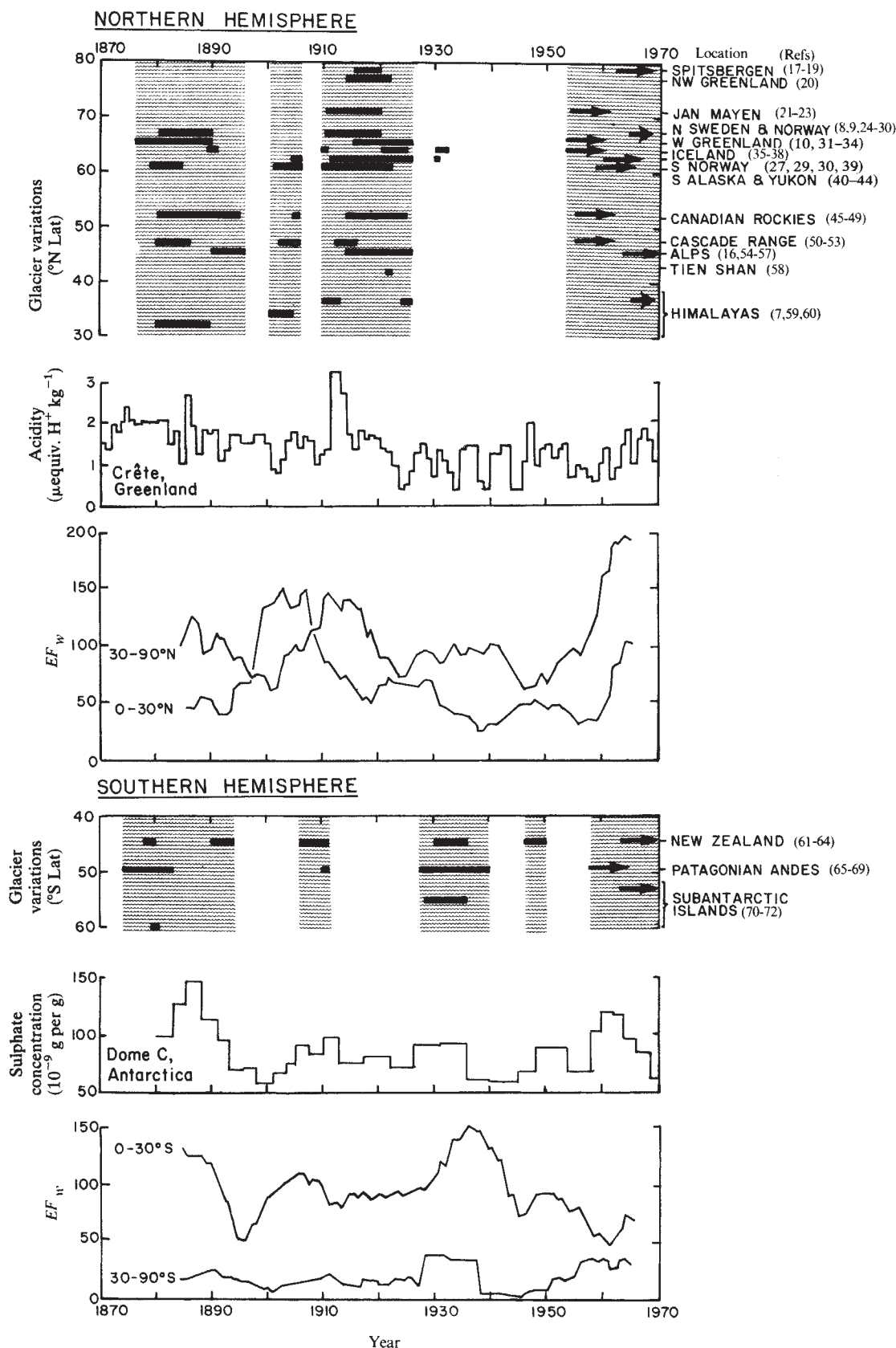


Fig. 2 Comparison of recent glacier variations in the Northern and Southern Hemispheres with acidity profiles from polar ice sheets and frequencies of recorded volcanic eruptions. Data sources for glacier variations given in parentheses. Bars represent intervals within which reported glacier advances culminated and are based mainly on direct observations; in a few cases they are based on ring counts of glacially tilted trees and by lichenometric dating where well-controlled lichen-growth curves have been constructed. Beginning of recent episodes of advance indicated by arrows. Data from known or suspected surging glaciers were excluded because marginal fluctuations of such glaciers may be anomalous and apparently unrelated to local or regional climate. Northern Hemisphere data set includes 115 culminations recorded for 76 glaciers, as well as 10 regional summaries, whereas Southern Hemisphere set represents 50 culminations for 30 glaciers. Spread of dates within each interval mainly reflects response lags of glaciers of different size or having different activity indices, or possible dating uncertainties. Vertically shaded zones show main groupings of data within each hemisphere. Acidity records from Crête (central Greenland) are from ref. 12, while Dome C (Antarctica) data are from ref. 13. Time series of weighted eruption frequencies (EF_w), based on compilation of Hirschboeck¹⁴, are 10-yr running totals.

glaciers that culminated in the late 1940s was not matched in northern latitudes where most glaciers continued to recede until the middle 1950s. This contrast in recent glacier fluctuations in the two hemispheres must be explained by any acceptable hypothesis of short-term climatic change.

A comparison of the hemispheric records of glacier fluctuations with variations in the concentration of volcanically-generated sulphate in polar ice sheet cores that has accumulated

over the same time interval discloses some striking parallels. Volcanic acids deposited in snow layers shortly after a large eruption can be detected by measuring specific conductivity of meltwater samples or by electrical measurements of solid ice. An acidity profile along the well-dated Grête ice core from central Greenland¹² has strong peaks in the middle 1880s and about 1912-15. Sulphate concentration in snow layers from Dome C in East Antarctica¹³ is high in the middle to late 1880s

and in the late 1950s to early 1960s, with secondary peaks about 1905–11, 1926–35 and 1948–53.

The high acidity levels in polar ice correspond to peaks in Hirschboek's weighted time series of the frequency of volcanic eruptions in low and high latitude belts of the two hemispheres¹⁴. The eruption frequency time series were constructed by classifying recorded eruptions by magnitude and conservatively assuming that great ash eruptions were an order of magnitude larger than moderate eruptions. Decadal running totals for the period since 1880 are based on more than 1,300 great and moderate events. The time series show that the late 1880s and early 1890s were volcanically active in both hemispheres, but that during the twentieth century the Northern Hemisphere experienced periods of high volcanic activity during the 1900s (especially at low latitudes), the 1910s (especially at middle and high latitudes) and in the late 1950s and the 1960s. Volcanoes in the Southern Hemisphere were active during the first decade of this century at low latitudes, but the major period of eruptive activity in both latitude zones occurred between about 1928 and 1940.

The two types of volcanic records each have shortcomings for analysis of the spatial and temporal significance of aerosol-producing eruptions. The existing ice-core records come from the polar regions (71.6° N and 74.7° S, respectively) and probably emphasize eruptive events at middle to high latitudes (although certain large low-latitude events like the 1883 eruption of Krakatoa (6° S) are recognizable in both ice sheets; the lower magnitude 1963 Agung event (8.5° S) is identified as a strong peak in the Antarctic core but is less pronounced in the Greenland record). The acidity curves may de-emphasize large low-latitude eruptions that generate little sulphate but eject fine ash in quantities sufficient to affect the climate for brief periods. The eruption frequency curves do not distinguish between ash-emitting eruptions and important sulphur-rich eruptions, the latter producing the most significant long-lasting atmospheric effects. Furthermore, the frequency variations must partly reflect varying observational frequency rather than varying volcanic activity. Nevertheless, the similar pattern in the two types of record over the past 100 yr suggests that they may provide a reasonable picture of the temporal and spatial distribution of major eruptions which have had the greatest impact on the atmosphere.

Episodes of glacier resurgence culminated during or shortly after intervals marked by high acidity levels in polar ice and increased frequency of volcanic eruptions in each hemisphere. The high eruption activity of northern middle- to high-latitude volcanoes in the 1910s and a corresponding acidity peak in Greenland ice, for example, coincided with widespread northern latitude glacier advances that culminated mainly between about 1912 and 1925, while high volcanic activity, a secondary acidity peak in Antarctic snow, and glacier re-advances marked the late 1920s and the 1930s in southern middle latitudes. The northern low-latitude peak in volcanic activity during the first decade of this century coincides with glacier advances in lower middle latitudes; such advances have not been reported from high latitudes. A similar pattern is seen in the Southern Hemisphere at the end of that decade. The resurgence of glacier activity in both hemispheres beginning in the middle to late 1950s parallels an increase in reported volcanic eruptions, as well as increases in acidity levels in the polar ice sheets.

The data base used in this analysis is of necessity restricted largely to middle latitudes. Moreover, the Southern Hemisphere data are less numerous than those from northern latitudes; this partly reflects the greater extent of land and of glaciated mountains in the Northern Hemisphere, but also a more thoroughly documented observational record. Further testing of the hypothesis, involving information from low-latitude glaciers, must await further data.

Extension of the analysis back in time is also desirable, but the number of carefully-documented glacier observations falls off rapidly in the decades before the middle nineteenth century, and the spatial distribution of data becomes much more restricted.

Moraine sequences offer a basis for reconstructing the pattern of earlier glacier variations, but they are likely to be incomplete, and the potential for dating error increases with age. For these reasons a comparable global assessment of glacier variations and volcanic activity for earlier periods may be less convincing. Nevertheless, the patterns observed here apparently do continue back at least into the late eighteenth century, and possibly before¹⁵, for recorded advances of glaciers in northern middle latitudes that climaxed between about 1770–90, 1814–25, and 1845–55¹⁶ match rises in ice-core acidity levels and in the frequency of great ash eruptions^{12,14}. These relationships, like those documented above, support the view that volcanic aerosols produced during episodes of large-scale eruptions are a major factor in modulating climate on the decadal scale.

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Glaciation, erosion and uplift over part of East Antarctica

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Isostatic rebound effects after the removal of large glacial loads are well known from several parts of the world, but isostatic uplift during glaciation and caused by glacial erosion is less well documented. This is because preglacial landscapes, which provide a datum against which glacial erosion and related uplift is measured, are generally either destroyed by glaciation or not recognized. Remnants of such a preglacial land surface are exposed in the Prince Charles Mountains of East Antarctica. We now report that the amount of glacial erosion here is 1.5 km, measured by the difference in level between the preglacial land surface and the present mean (mostly subglacial) rock surface. The isostatic uplift caused by this erosion is ~0.8 km. Both amounts are thought to be well above average for East Antarctica and glaciated areas in general because the region is traversed by East Antarctica's largest outlet glacier system, parts of which are believed to surge periodically.

The Prince Charles Mountains (72°S, 72°E) are composed of complexly deformed Archaean and Proterozoic igneous, sedimentary and metamorphic rocks which are intruded by Cambrian granites and locally overlain by small intermontane enclaves of Permian sedimentary strata¹. Near the main glaciers of the Lambert Glacier system the rocks are exposed in large mountains and massifs with broad, roughly concordant, summit plateaus; away from the glaciers there are long low ridges or small nunataks with spiky summits at the same or a lower level².

Topographical data³ and field and airphoto studies have shown that summit altitudes range systematically from 1.25 km in the centre of the area—where summit plateaus are very flat and have 0.2 km of relief—to 2.60 km at the margins where summits, though roughly concordant, are of less uniform altitude (Fig. 1; Fig. 2 curve a). This has led to the interpretation, at first based mainly on morphological grounds, that the summits are relics of a preglacial erosion surface of generally low relief and locally nearly planar. A surface along the flanks of the Amery Ice Shelf with a present altitude of 0.2 km (Fig. 1 stippling) may be a downfaulted continuation of the erosion surface exposed further south. Remnants of nearly planar erosion surfaces of pre-breakup age are widespread in various Gondwanaland fragments^{4–6} and we believe that the erosion surface exposed in the Prince Charles Mountains is of the same origin and age.

The summit plateaus in the Prince Charles Mountains, although regarded as the relics of a preglacial erosion surface have, nonetheless, been glaciated and have local mantles of exotic glacial debris and striated pavements. At one locality, however, (Fig. 1D) an erosion surface on banded gneisses is unconformably overlain by a partly vesicular olivine leucite flows that gives a K–Ar whole-rock age of 50.4 ± 2.0 Myr (ref. 7). The 3×1 km flow remnant has vertical sides standing up to 10 m above the adjacent erosion surface. From the existence of this Eocene flow we infer that the underlying erosion is of pre-Eocene and therefore of preglaciation age, and that the

glaciation has caused only minor erosion of the erosion surface on which it lies. This minor glacial erosion is thought to be areal scouring by a relatively thin ice cover with basal temperatures below the pressure melting point^{8,9}.

The present mean level of the preglaciation surface is shown as a' in Fig. 2; the difference between it and the present mean level of the regional rock surface is a measure of the glacial erosion that has taken place. Subglacial rock altitudes derived from ice thickness surveys^{10,11} show that the present rock surface has a relief of up to 4 km. They are used together with the altitudes of exposed rock masses in estimation of the present mean rock surface level for 50×50 km areas. These in turn were used to calculate a running mean of rock altitudes over 100×100 km areas (Fig. 1; Fig. 2, curve e). This mean rock surface (curve e) ranges from -0.5 km along the axis of the lower Lambert Glacier to +0.5 km at the margin of the area studied. The difference in level between it and the mean preglaciation surface (Fig. 2, curve a') ranges from 1.3 to 2.0 km with a mean near 1.5 km; it is a measure of the regional glacial erosion.

Erosion of this order is likely to have caused vertical movement of the crust in the Prince Charles Mountains area although this will have been counteracted by the effect of the present glacial load. The low relief of the preglaciation land surface is interpreted as evidence that the area was stable in relative altitude and hence likely to have been in isostatic equilibrium before glaciation. We concluded¹² that the area is now in

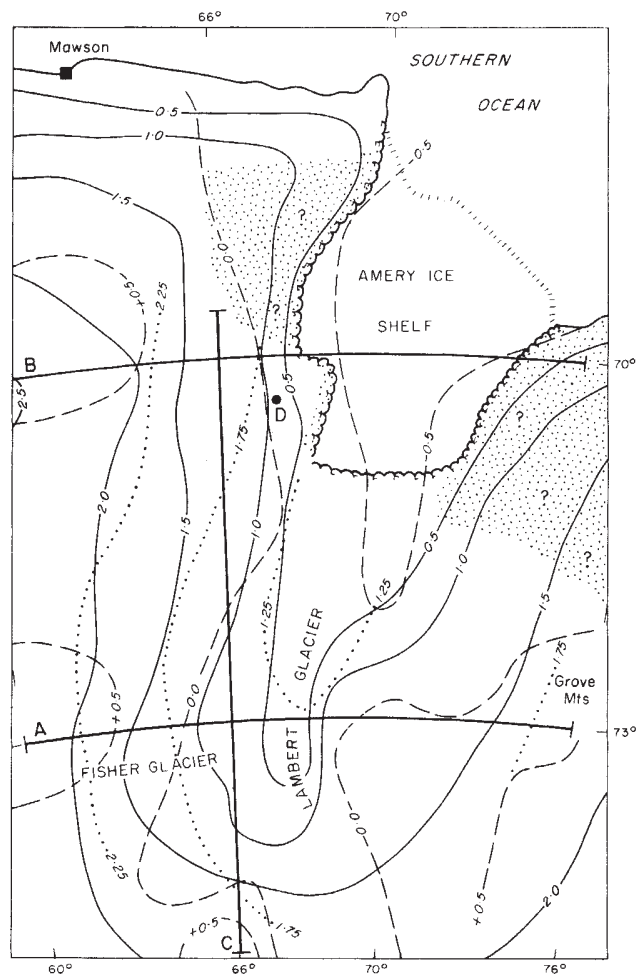


Fig. 1 Map of the Prince Charles Mountains area showing the present-day altitudes in kilometres of the high points of the summit plateau surface (dotted lines), the mean rock surface (dashed lines) and the ice surface (solid lines). Thick lines show the location of Fig. 2 cross-sections. D is the location of the Eocene lava flow discussed in the text. The stippled area is a possible downfaulted portion of the summit plateau surface, now at 0.2-km altitude.

isostatic equilibrium from a previous analysis of mean rock altitudes, ice thicknesses for 100×100 km areas, and gravity data. If crustal structure does not change, then the removal by erosion of rock masses from a crust in isostatic equilibrium results in an uplift of the crust to maintain this equilibrium. The magnitude of this uplift depends on the density contrast between the crustal rocks and the underlying upper mantle¹³; it is likely to be between 0.80 and 0.87% of the thickness of rock removed. If there were no present-day ice load the original level of the preglaciation peneplain surface could be calculated to have been above the level of the present regional mean rock surface by 0.13–0.20 times the thickness (1.3–2.0 km) of rock removed by glacial erosion, or 0.17–0.40 km.

However, the present mean rock surface (Fig. 2, curve e) is depressed by ice loading, the effect of this loading being the same as that of a rock layer with the same mass per unit area as ice, that is 0.27–0.29 times the ice thickness (Fig. 2, curve b minus curve e) or between 0.3 and 0.5 km. On deglaciation the mean rock surface would be at the level of curve d of Fig. 2. The original level of the preglaciation peneplain would therefore have been 0.17–0.40 km above the level of curve d, at the level of curve c. It would have ranged up to ~1 km altitude near the edge of the region studied. The amount of isostatic uplift that has already occurred as a consequence of glacial erosion is given by the difference between the present level of the mean preglaciation surface (Fig. 2, curve a') and its original level (curve c). It is between 0.7 and 1.5 km with a mean of 0.8 km.

This uplift and both the rate and amount of glacial erosion that caused it are thought to be above average for East Antarctica and glaciated areas in general because:

- (1) The area is traversed by the largest ice stream in the world—there is a large ice flux from a large catchment.
- (2) Glacier velocities are high because the area is near the maximum slope of the Antarctic ice cap and the lower sections of the glaciers have basal melting.
- (3) At least part of the glacier system may be subject to periodic surging, with consequent short-lived episodes of very vigorous erosion.

However, the relative size of the present-day and past erosion rates is unknown. Modern evidence for near-surface glacial erosion in the Prince Charles Mountains is not abundant and consists of local undercutting of steep cliffs by locally steep, heavily crevassed glaciers. The major ice streams that traverse the Prince Charles Mountains have a base at or near pressure melting point¹⁴, so significant subglacial erosion may be occurring^{8,9}. However, it is possible that the valleys are now sufficiently deep that there is no significant erosion at the present flow rate. It seems unlikely that much of the erosion took place during the alpine glaciation that preceded the continental glaciation in the Miocene, because we believe that at this time relief and altitude were low in the Prince Charles Mountains area. It is more likely that erosion along the ice streams has continued during the whole period of continental glaciation but, except initially, has been intermittent and largely related to periodic surging of the glaciers. Such surging is suggested by glaciological studies^{14,15}, and also from an interpretation of moraines between 0 and 200 m above the present ice level of the Fisher and lower Lambert Glaciers¹⁶. Normal movement of large glaciers at rates between 50 and 200 m yr⁻¹ is achieved by ice shear and recrystallization within the ice mass. During short-lived periodic surges the glacier movement and erosion are several orders of magnitude greater, the fast movement being by the ice moving on a basal water layer.

Evidence for high erosion uplift rates is likely to be found elsewhere in East Antarctica where major glaciers such as the Jutulstraumen traverse mountainous areas near the maximum slope of the ice cap. In mountainous areas of Greenland and north Canada, on the other hand, erosion and erosion-induced uplift are probably occurring more slowly because the glaciers are smaller and zones of high glacier slope are narrower. Nonetheless, the glacial landscapes of the mountains of Greenland and north Canada^{8,9} are strikingly similar to those

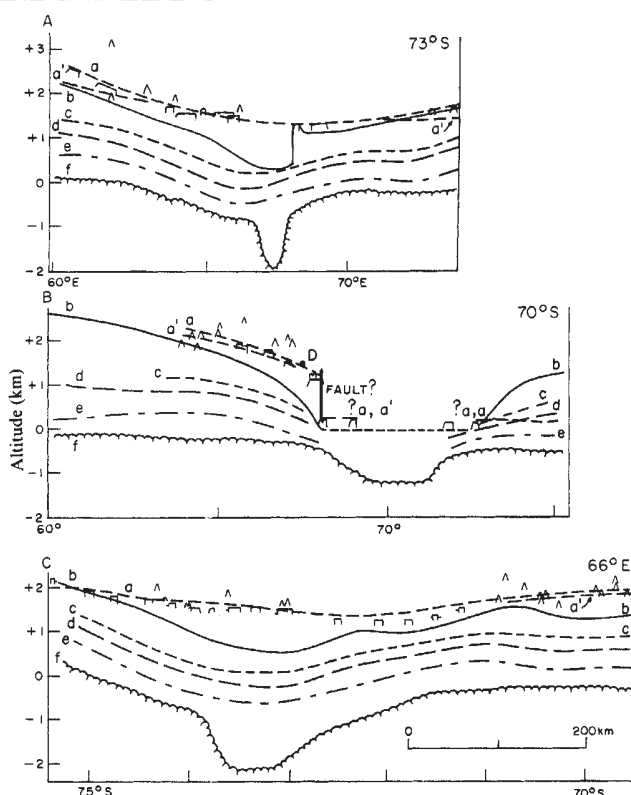


Fig. 2 Sections showing: projected high points of summit plateau and spiky summits, and their mean level (curve a); the present level of the mean preglacial surface (curve a'); the present ice surface (curve b); the original level of the preglaciation surface (curve c); predicted mean level of the present rock surface after deglaciation (curve d); present mean rock surface (curve e); and present lowest rock surface within 100 km of the section line (curve f). Fig. 1 shows the location of these sections.

found in the Prince Charles Mountains and are classified as landscapes of selective linear erosion.

Erosion rates beneath the main East Antarctic ice cap are probably much lower than those found in the Prince Charles Mountains because of the much lower ice velocity, much less widespread basal melting and lower rock relief. The subglacial landscape in these areas is likely to be one of areal scouring similar to that found in areas of Canada, Greenland and Fennoscandia which are thought to have been once covered by major ice caps¹⁷. The Fennoscandian and Canadian shields are thought to have been glacially eroded by only a few tens of metres¹⁸.

The average rate of erosion inferred for the Prince Charles Mountains region since the onset of glaciation in the Oligocene¹⁹ is 1.5 km during 30 Myr or 0.05 km Myr⁻¹. This is lower than reported rates²⁰ for very active orogenic areas: 0.21 km Myr⁻¹ for the Himalayas during the past 40 Myr and 0.12 km Myr⁻¹ for the Rocky Mountains in the late Cretaceous. It is similar to erosion rates for slightly orogenic areas such as the Appalachians or the Mississippi Basin. Our observations in the Prince Charles Mountains region thus apparently confirm that continental glaciation is not an especially vigorous erosional agent. Furthermore, although the Antarctic continent is widely regarded as being depressed by the ice load there are areas where glacial erosion has induced a net local uplift.

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Short-term fluctuations in heavy metal concentrations in Antarctic snow

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Heavy metal variations in polar ice cores may reflect changes in global airborne pollution¹. Reported profiles from Antarctica¹⁻³ and Greenland⁴, where sampling resolution was normally larger than a year, have shown a much greater variability than can be reasonably accounted for by variations in the annually smoothed emissions of anthropogenic sources and most natural sources. Volcanism has been invoked⁴ to account for major features. We now report data from a finely resolved sample sequence from a remote plateau region of the Antarctic Peninsula. Variations of similar magnitude to those reported for longer time series occur also on the scale of seasonal changes and even of single snowfalls; these must be controlled by meteorological processes. We conclude that changes in large-scale transport processes and depositional mechanisms on longer time scales may have as important a role as emission rates in generating the concentration profiles observed in deeper ice cores.

Snow samples were collected in January 1977 from a remote site on the Antarctic Peninsula plateau (70°53'S, 64°57'W; altitude 1,860 m), 170 km from the Weddell Sea to the east and 200 km from the Bellingshausen Sea to the north-west. The plateau at these latitudes is 60-90 km wide and bordered east and west by mountain ranges, though neither was closer than 40 km. The prevailing northerly winds came from an area generally free from exposed rock.

Samples for heavy metal analysis were collected from a clean zone 150 m upwind of a vehicle-free camp site. All sampling tools and containers had been given three lengthy washes in 1:6 HNO₃ (Analar) followed by two thorough rinses with ultrapure water in the laboratory before transportation to the sampling site in sealed polythene bags. Two investigators, wearing full clean room garb, dug a 2-m-deep pit with steel shovels and then trimmed 10 cm from the rear wall using a polyethylene scraper.

Triplicated pit-face samples and duplicated 2-m cores (9-cm diameter) recovered directly behind the face with an aluminium hand auger were subdivided with a PTFE saw and stored in 1-l polyethylene containers. Relatively large and erratic metal concentrations subsequently measured in these samples suggested that contamination had occurred, probably from the

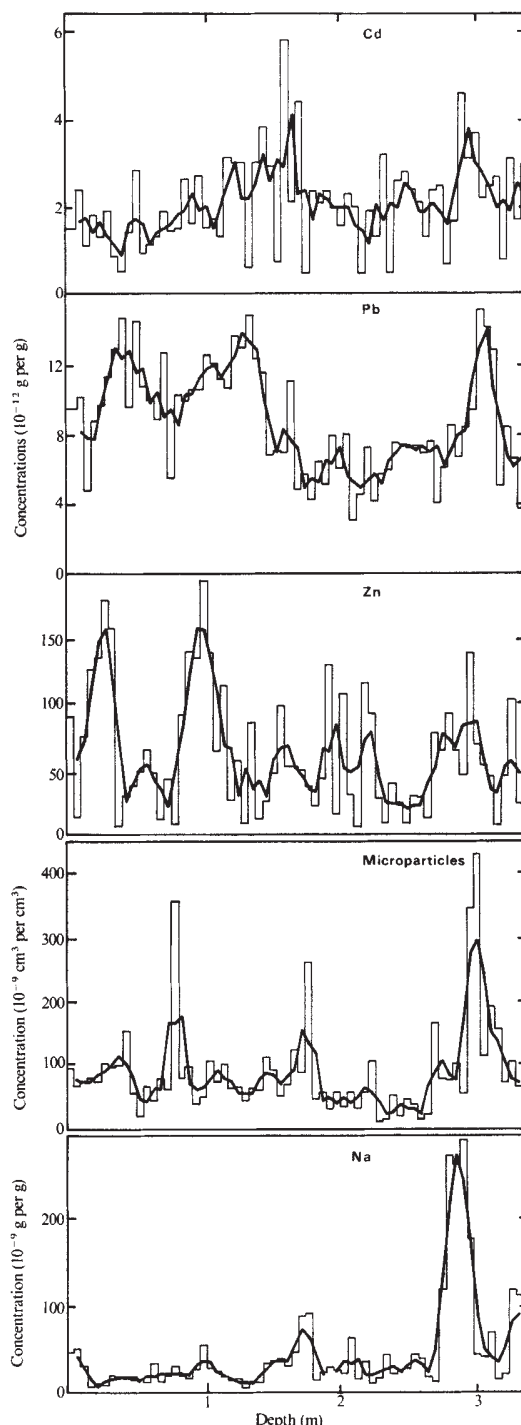


Fig. 1 Concentration profiles for Cd, Pb, Zn, microparticles (0.5-1.8 μ m) and Na in the upper 350 cm of snow (May 1973-January 1977). The bold lines are running averages of three data points.

PTFE saw. However, a 3.4-m core in ~20-cm lengths inside 60-cm acrylic tubes with polyethylene caps gave consistent data with no evidence of contamination; these values are presented here. For the marine ion and microparticle analyses, a 6.5-m core was sealed into polyethylene sleeving and placed inside 100-cm cardboard tubes. The cores were all collected within an area 30-cm square and stored at temperatures below -10 °C before analysis.

Analyses for Cd, Pb and Zn were performed on snowmelt in a Class-100 laminar flow work station by differential pulse anodic

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stripping voltammetry (DPASV) at a rotating glassy carbon electrode with *in situ* mercury plating⁵. The segments of the core stored in acrylic tubes were carefully transferred as required into a precleaned polyethylene container kept at -2°C . The core was briefly removed from its container with insulated PVC clean room gloves. Using a polyethylene scoop, snow was excavated 5 cm into the core, leaving a cylinder of at least 1 cm thickness around the exterior to exclude any superficial contamination. Approximately 30 g of the snow was transferred to a glass voltammetry cell, 50 μl of a mercuric nitrate in 5% HNO_3 plating solution was added (giving $2 \times 10^{-5} \text{ M Hg}^{2+}$ at pH 2.9) and the sample was melted immediately before analysis. The quantity required for DPASV allowed almost continual analysis every 5-cm down the core, sufficient resolution to permit the study of short-term variations in concentration.

Three DPASV runs were performed on each sample, the final one with a standard addition of 1.5 ng Cd, Pb; 3.0 ng Zn, Cu (15 μl of $10^{-7} \text{ g per g Cd, Pb; } 2 \times 10^{-7} \text{ g per g Zn, Cu}$ in 1% HNO_3). The exact volume of the sample was measured after analysis. Due to the difficulty both of obtaining ultrapure water with heavy metal concentrations consistently smaller than the cleanest snowmelt analysed and of using this to simulate a snow analysis, the concentrations presented here are not corrected for a procedural blank, and as such represent upper limit values. As one core only was available for heavy-metal analysis it was not possible to make a comparison with duplicated samples treated by more complete acid digestion. In a related study at Antarctic sites much closer to exposed rock, we have found⁵ that digestion at pH 2.9 for 4 h at 110°C causes no perceptible increase in the observed heavy-metal concentrations except for samples taken very close to and down-wind from rock exposures. Sodium was measured in snowmelt at pH 1.5 by flame emission spectrometry, and the microparticle distribution above $0.5 \mu\text{m}$ was measured in a clean air laboratory using a Coulter counter.

Table 1 Correlation factor matrix, calculated from 3-point running averages

	Cd	Pb	Zn	D*	Na
Cd	1.0				
Pb	-0.03	1.0			
Zn	-0.10	0.29	1.0		
D*	0.19	0.32	0.12	1.0	
Na	0.45	-0.18	0.06	0.32	1.0

*Microparticles in the range $0.5\text{--}1.8 \mu\text{m}$.

The data from the top 3.4 m of snow (66 samples) are summarized in Fig. 1. The overall precision estimated from standards is 20% at the 95% confidence level. Superimposed on the data is a running average of three consecutive values, which reduces any systematic error introduced by comparing data from two different cores where identical snow layers may suffer a relative vertical displacement of up to 10 cm. The annual snow accumulation estimated from both stratigraphic observations and the oxygen isotope ratio profile of a 7-m core taken 100 m from the sampling site was 1.1 m ($48 \text{ g cm}^{-2} \text{ yr}^{-1}$). Support for the stratigraphy is obtained from spectral analysis of the marine-ion and dust concentration profiles (Fig. 1) which show strong peaks in auto-covariance and cross-covariance with lags of 115 cm. A seasonal periodicity for these species has been reported in other parts of Antarctica^{6,7}.

The average heavy metal concentrations over the 3-yr sequence are ($\text{Cd} = 2.1 \pm 1.0$, $\text{Pb} = 8.8 \pm 3.1$ and $\text{Zn} = 63 \pm 47$) $\times 10^{-12} \text{ g per g}$. Cu concentrations were usually near or below the detection limit; however, an upper limit of $20 \times 10^{-12} \text{ g per g}$ was obtained as an average over 35 samples. These values are similar in magnitude to those published for much more remote sites in Antarctica (Dome C¹ and South Pole²), and an order of magni-

Table 2 Crustal heavy metal enrichment factors calculated from the 3-point running averages in comparison with averages for east Antarctic snow deposited since 1968^{1,2}

Crustal	Average	Minimum	Maximum	Comparative values for east Antarctic snow
Cd	52.6	14.2	131.5	4,400
Pb	3.5	0.9	7.6	160
Zn	4.0	0.7	12.8	40

tude smaller than concentrations measured by DPASV on the less remote Adelaide Island (Antarctic Peninsula)⁵. Low concentrations could be the result of a much larger snow accumulation rate at this site compared with the remote sites in East Antarctica, although there is evidence that trace element concentrations in snow are independent of the snow accumulation rate⁸.

Previous studies have used more complete digestion of samples before analysis by flameless AAS and therefore these data may contain an additional proportion of metal species not included in our analysis. Our data refer to metal available to DPASV at pH 3: free metal ion, labile complexes, metal dissociated at the electrode surface from complexes and colloids, and species adsorbed on mineral matter. From the scant evidence available on the nature and reactivity of heavy metal particulates in the atmosphere, it seems likely that our analysis includes aerosols from the major pollutant sources which include mining, base-metal smelting and refining, and automobile exhausts; these are predominantly oxides, carbonates,

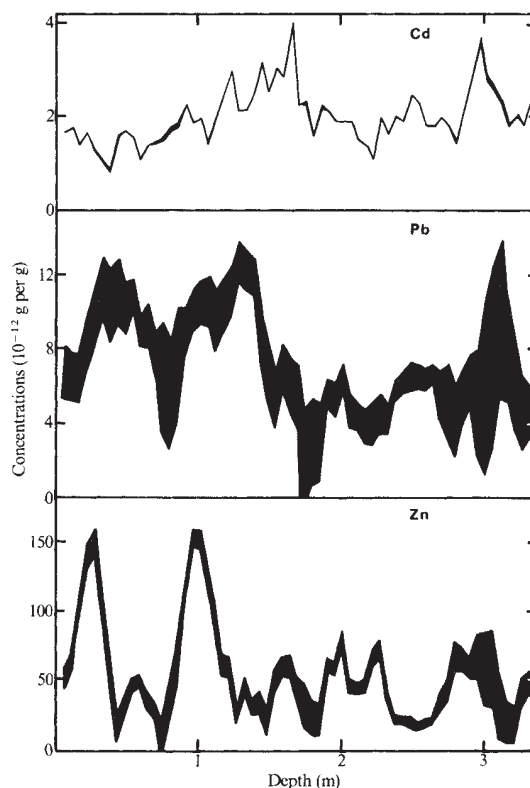


Fig. 2 Microparticle contribution to the heavy metal concentration profiles. The shaded area represents the proportion of metal attributable to microparticles, calculated using average crustal abundances¹².

sulphides, halides and elemental⁹. Emissions of elements and simple compounds by volcanic activity, postulated as a significant source of globally circulating heavy metals^{1,3}, would also be analysed, along with products of biological processes such as methylation which have been proposed as important natural sources of trace metals in the atmosphere^{10,11}. Such emissions would be degraded by photochemical activity during long range atmospheric transport, and probably converted into oxide. Our analysis excludes the contribution from wind-blown dust where heavy metal has replaced other elements in a silicate lattice. Thus we would expect to exclude much of the heavy metal associated with mineral particles derived from aeolian action on the exposed volcanic rocks of the Antarctic Peninsula, which are likely to dominate the mineral content of our samples. The mean dust volume (m^3 per m^3 snowmelt) concentration at our sampling site is 110 p.p.b. (parts per 10^9) which can be compared with a concentration of 24 p.p.b. in the same size range reported¹² for the mean of snowfall at the South Pole deposited immediately before the recent increase in dust concentration at this site due to local human activity¹³.

This value may be considered the upper limit to the concentration of microparticles transported to the Antarctic continent from neighbouring continents. On this basis, we estimate that ~80% of the microparticles in our samples are of local origin. If these make a significant contribution to the heavy-metal concentrations observed, then some correlation between the depth profiles for the heavy metals and the microparticle concentrations might be expected. The correlation matrix in Table 1 shows that, with the exception of a weak correlation (significant at the 5% level) between Pb and microparticles which arises from the coincidence of a single major peak at about a depth of 3 m, there was no correlation between the profiles despite the relatively large mineral content.

We can estimate the form of the maximum possible contribution by local dust to the input of heavy metals by assuming that these are of mean crustal composition and that all the heavy metal is available at pH 3. In estimating the mass flux we consider particles in the size range 0.5–1.8 μm . This upper limit prevents a bias in the data due to the influence of a relatively small number of larger particles which are least likely to be available for DPASV analysis. On the average, this size range represents ~53% of the total particle flux between 0.5 and 6 μm diameter. Figure 2 shows how the concentration profiles are modified when allowance is made for the contribution from local dust. The procedure seems to overestimate the contribution because for certain discrete events the estimate of the local heavy metal component is slightly greater than the total amount analysed. Except for occasional periods where trace element concentrations are low and may be almost completely of local origin a maximum average of only 2% of Cd, 29% Pb and 25% of Zn could be attributed to local dust. The residual heavy metal which we interpret as a long-range input to the Antarctic environment, shows enhanced short-period variations.

We have calculated crustal enrichment factors for the heavy metals (Table 2) assuming that the microparticles are of mean crustal composition¹⁴

$$EF_{\text{crust}} = \frac{[\text{metal}] \text{ snow} / [\text{microparticles}] \text{ snow}}{\text{proportion metal in average crustal material}}$$

These values represent upper limits as our estimate of microparticle concentration is based on a restricted size range, yet they are between 10 and 80 times lower than enrichment factors calculated for snow in east Antarctica^{1,2}. These previous studies have calculated enrichment factors using Al as a crustal reference element, Al being measured¹⁵ in samples of snow melt before digestion for heavy metal analysis. Comparison of the two methods is possible at the South Pole, where Hamilton and O'Kelly¹² measured microparticle size distributions using a Coulter counter in a continuous sequence of samples from a shallow pit covering the period 1956–64. From these data, the

authors estimated a mean total dust volume concentration of 24 p.p.b. (72 p.p.b. by weight). This probably underestimates microparticle concentration as particles smaller than 0.5 μm are excluded. If the dust had contained a normal crustal abundance of Al then Al concentrations should have averaged 5.9 p.p.b. For samples covering the same period Boutron *et al.*² report direct measurements of 1.5 p.p.b. Al. Therefore, either previous reports represent samples in which Al may have been incompletely analysed due to partial sedimentation of mineral microparticles whilst the samples were melted, or there is considerable error in assuming that the average composition of microparticles penetrating the Antarctic environment is mean crustal in the major elements. This discrepancy could account for a reduction of our EF_{crust} values by a factor of 4, and a further factor of 5 could be due to the previously mentioned dilution effect of local dusts.

Although some of the noise in our concentration profiles may represent variations occurring during individual snowfall events due to progressive scavenging of impurities from the atmosphere, the depth range occupied by some of the major peaks is large compared with the likely precipitation in a single event. Moreover, there is a substantial difference, most noticeable for Zn and Pb, between the mean concentration in the upper 1.5 m and that in the lower 1.5 m on the time scale of seasonal and year-by-year changes. These variations, which range over an order of magnitude for Zn to a minimum factor of 2 for Cd, are much larger than can reasonably be accounted for by variations in global emission to the atmosphere. Sharp variations of similar magnitude, although with a much coarser resolution in longer time scales, have been reported in sample sequences from both the Antarctic and Greenland ice sheets. Several processes, including volcanism^{1,3,4,9} have been invoked as possible causes of sporadic variations in global emission rates into the atmosphere.

In support of this idea a 100-yr old sample sequence from central east Antarctica has shown³ a close parallel between major peaks in the stratospheric burden of volcanic aerosols and the measured concentrations of Zn and Pb. However, a similar mechanism cannot be invoked to account for the shorter term variations observed in our samples which are evidently linked to meteorological transport and depositional processes. As these seem to be capable of generating features in the concentration profiles preserved in snow layers in the short term which are of comparable magnitude to the variations observed in the longer records, we conclude that longer term changes in related meteorological processes may play a significant part in shaping the corresponding record in snow which need not necessarily reflect changes in global emission rates. Until the mechanisms responsible are more fully investigated caution must be exercised in interpreting variations in the concentrations of trace elements in ice cores in terms of varying global emission rates.

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Age of mineral equilibria in granulite facies nodules from kimberlites

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Among the nodular xenoliths erupted with kimberlites and alkali basalts some plagioclase-bearing and other granulite-facies nodules with metamorphic textures may represent rock samples plucked directly from the Earth's lower crust and Moho region. In studying these xenoliths it is tempting, as in the case of deeper mantle xenoliths¹, to use temperature–pressure estimates from mineral compositions to reconstruct petrological–depth relationships and geotherms for the time of eruption.^{2–4} In such reconstructions it has been stressed^{5–9} that possible modification of the xenolith mineral equilibria during the processes of eruption must be considered; but we emphasize here that the reconstructions may also be invalid because mineral equilibria had become inert or frozen long before eruption. We pay particular attention to nodules from kimberlites in Lesotho, and present some new age data. We argue further that medium- and coarse-grained rocks lacking a fluid phase in stable tectonic regions are unlikely to maintain local equilibrium at ambient geotherm conditions until depths well within the upper mantle are reached.

Granulite-facies xenoliths are relatively abundant in post-Karoo kimberlites occurring close to the supposed position of the southern margin of the Kaapvaal craton in South Africa and Lesotho⁴. Clinopyroxene, garnet and plagioclase are the common major minerals of the xenoliths, with orthopyroxene less common, and amphibole, biotite and scapolite relatively scarce. The nodules often show granuloblastic textures. Rogers and Du Toit¹⁰ noted that the rocks of the nodules were probably related to Proterozoic granulites (of the Namaqua–Natal belt) exposed near the margin of the Kaapvaal craton. Griffin *et al.*⁴ partly endorse such a relationship, but they relate the granuloblastic textures to annealing caused by heating during the Karroo episode, and consider the mineral equilibration temperatures to represent deep crustal temperatures at the time of kimberlite eruption. Betton and Cox (see refs 11, 12) suggest that the rocks represented by the nodules were initially formed adjacent to the crust–mantle boundary by crystallization of Karroo magmas and that they reequilibrated in deep crustal conditions before kimberlite eruption.

Recent work^{3,4,13–15} has provided a relatively large amount of mineralogical data for estimating temperature–pressure conditions of formation for the granulite-facies nodules from northern Lesotho. For the plagioclase-bearing assemblages: Griffin *et al.*⁴ estimate temperatures predominantly between 550 and 770 °C with pressures spreading largely from 4.5 to 15.5 kbar; Jackson¹⁵ estimates temperatures and pressures in the ranges 600–800 °C and 15–20 kbar. These temperature–pressure estimates yield unexpected results when used to reconstruct the thermal and petrological characteristics of the crust–mantle section underlying Lesotho at the time of kimberlite eruption. The temperatures, except in combination with the highest-

pressure estimates, are distinctly higher than those expected by comparisons with normal geotherms for Precambrian shields and platforms and present data for southern Africa^{16,17}. Also the higher pressure estimates¹⁵ clearly place plagioclase-bearing rocks within the present day mantle as defined by available seismic data¹⁸ on the depth of the Moho in South Africa.

Assuming that the nodules reflect ambient crustal temperature–pressure conditions at the time (Cretaceous) of kimberlite eruption, Griffin *et al.*⁴ attribute the high temperatures estimated to the slow decay of the thermal effects of the earlier Karroo volcanism, and postulate a Cretaceous geotherm with several inflections. We suggest that the possibility must be considered that rocks in the lower crust contain frozen mineral equilibria, which, in this case, may be relics of Proterozoic orogenesis or of high geothermal gradients at the actual time of Karroo magmatism. Although the temperatures of 550–800 °C estimated from the granulite-facies nodules are high for stable continental crust they are by no means high compared with those indicated by amphibolite- and granulite-facies rocks in exposed orogenic belts. Traditionally, rocks collected at the Earth's surface from exhumed orogenic belts are assumed to contain frozen mineral equilibria predominantly reflecting the highest temperatures (or highest entropy states¹⁹) reached by the rocks. This assumption, which is applied to both volatile-bearing and volatile-free assemblages, is essentially the opposite of that of presumed approximate continual equilibration at depth adopted for the granulite facies nodules in the reconstruction of geotherms for the time of eruption.

The relatively dry nature of the nodule mineral assemblages from Lesotho as shown by the restricted occurrence of primary hydrous minerals (amphibole and biotite)^{4,14,15}, together with their estimated temperatures of formation, must indicate metamorphism in conditions of highly restricted water content²⁰. Where such 'dry' rocks are subjected to metamorphism involving little deformation in the temperature range indicated for the nodules then the products usually fail to approach general textural and mineralogical equilibrium on the scale of a thin section—typically original textures and compositions are partly preserved whilst minerals are clouded and show partial replacement and corona textures^{21–24}. Such features contrast with the general appearance of textural and mineralogical equilibrium seen in the Lesothon granulites^{4,13–15}, and cast doubt on them having equilibrated to ambient geotherm conditions since their initial formation.

We have endeavoured to obtain direct evidence on the age of the mineral equilibria in the nodules by radiometric dating. Two ²⁰⁶Pb–²³⁸U ages previously obtained²⁵ for zircons from the Lesothon granulite xenoliths yielded Proterozoic ages of 1,500 and 1,050 Myr. However, the refractory nature of zircon makes it unlikely that it reflects the time of recent equilibrium in most minerals. Biotite and amphibole are present in some of the nodules and the relatively low blocking or closure temperatures for Ar accumulation imply that the ⁴⁰K–⁴⁰Ar method on these minerals provides a means of assessing the minimum age of the mineral compositions yielding the temperature–pressure estimates. We have found only two nodules with sufficient fresh and texturally primary amphibole or biotite for separation and analysis. The K–Ar analytical results for the minerals from these nodules are given in Table 1: the mica yields an apparent age of ~1,000 Myr and the amphibole 714 Myr.

These Proterozoic ages clearly far exceed the <100 Myr ages measured for emplacement of southern African kimberlites²⁵. The two ages may relate to different geological events, but it would have been more reassuring had they been consistent. Furthermore the incorporation of extraneous ⁴⁰Ar which leads to erroneously old ages is well documented in some metamorphic micas²⁶ including, in particular, those from granulite facies rocks²⁷. However a K–Ar whole-rock age of 90 Myr for the Monastery kimberlite²⁸ agrees with the Rb–Sr and U–Pb ages^{25,29} and therefore does not suggest the presence of any extraneous Ar in the kimberlite. Also the 1,000 Myr biotite age agrees well with the 1,050 Myr obtained for one of the zircons

Table 1 K–Ar analyses of minerals from Matsoku Pipe, Lesotho

Sample no.	K (wt %)	$^{40}\text{Ar}^*$ ($\times 10^{-4} \text{ cm}^3 \text{ g}^{-1}$)	$^{40}\text{Ar}^*/^{40}\text{Ar}_T$	Age† (Myr)
Biotite (LBM 70)‡	7.72	4.063	0.988	1,010 ± 20
		4.013	0.989	1,000 ± 20
Amphibole (LBM 43)§	1.31	0.4463	0.974	714 ± 14
		0.4468	0.978	714 ± 14

* ^{40}Ar = radiogenic argon – 40; $^{40}\text{Ar}_T$ = total argon – 40.

† $\lambda_B = 4,962 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_K = 0.581 \times 10^{-10} \text{ yr}^{-1}$;

$^{40}\text{K} = 1.167 \times 10^{-4} \text{ atom \%}$.

‡ Mineral assemblage: garnet–clinopyroxene–biotite–rutile.

§ Mineral assemblage: garnet–orthopyroxene–amphibole–biotite.

extracted from the nodules²⁵, and is further supported by the extensive occurrence of ~1,000 Myr ages in the Namaqua–Natal Mobile Belt^{30–32} on the borders of the Kaapvaal Craton. However, an Rb–Sr measurement on biotite LBM70 (Table 1) yielded ages ($\lambda_{\text{Rb}} = 1.42 \times 10^{-11} \text{ yr}^{-1}$) of 178 and 200 Myr with assumed initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.710 and 0.704 respectively.

Amphibole has recently been used quite satisfactorily to date granulite facies metamorphism³³ and extraneous Ar is relatively unknown in this mineral; so, although the 715 Myr age measured on LBM43 is younger than those normally reported for the Namaqua–Natal belt, it could, nevertheless, reflect a Proterozoic event. It is similar to a zircon age of 650 Myr reported from an inclusion in kimberlite from further north-west in Swaziland²⁵ and to a K–Ar age of 728 Myr measured on primary mica from a peridotite nodule in the Monastery Pipe (R. M. Macintyre and J. B. Dawson, unpublished data). Further data are obviously desirable, but the available radiometric ages clearly suggest that the mineral compositions of Lesothon granulite-facies nodules are more likely to relate to Proterozoic events than the ambient temperature–pressure conditions just before kimberlite eruption.

Considering a section of lithosphere, which is not undergoing significant deformation, magmatic or hydrothermal fluid transport events, the question arises of at what temperature and depth along the geotherm are minerals capable of maintaining chemical equilibrium within reasonably short geological time periods (~10 Myr). More information concerning the time taken for minerals to equilibrate at particular temperatures and other conditions (for example, volatile abundances, amounts of strain) has long been considered desirable. In the present instance, with predominantly anhydrous and unstrained minerals of medium-coarse grain size, equilibration must involve volume diffusion and be relatively sluggish. The general petrologic evidence noted above clearly indicates that the temperature required to produce compositional and textural equilibration in such assemblages must be >700 °C. On the basis of petrological data, Yardley³⁴ suggests that the diffusion of divalent cations in garnet becomes relatively rapid at ~700 °C; but the common occurrence of Al and Cr inhomogeneities in garnets in garnet–peridotite nodules indicates that general equilibrium requires higher temperatures. Other evidence, associated with exsolution in coarse mineral grains, also indicates the probability of long-term disequilibrium in mantle nodules^{35,36}. Using approximate calculations³⁷ for diffusion distances and rates, the recent experimental data on Ca diffusion in clinopyroxene³⁸ indicates that temperatures >850 °C are required to equilibrate spherical grains of 5 mm diameter in 10 Myr. Hofmann and Hart³⁹ note the difficulty of achieving local equilibrium in crystalline peridotite lacking a melt phase at 1,000–1,200 °C; whilst comparisons^{8,40} of cation distributions and geothermometers for minerals from peridotite nodules may indicate significant limits to attainment of overall equilibrium by diffusion as temperatures drop to 1,100 °C.

With geotherm temperatures higher than 800 °C and possibly in excess of 1,000 °C necessary for equilibration of essentially volatile-free, magma-free and unstrained mineral assemblages

in a tectonically stable lithosphere section, there is a need to reconsider several aspects of the interpretation of upper mantle lithology and their bearing on magma genesis³⁹. Using a temperature of 900 °C as a minimum for equilibration by volume diffusion, the geotherms of Clark and Ringwood⁴¹ indicate the persistence of frozen mineral assemblages to lithosphere depths of 60 km beneath ocean basins and 105 km beneath continental shields. It follows that the 'dry' mineral assemblages adjacent to the Moho both in the lower crust and in large tracts of the upper part of the upper mantle cannot be predicted as usually assumed (see ref. 42) by a knowledge of phase equilibria, bulk composition and stable geotherm temperature–pressure estimates. Instead the mineral assemblages will be largely a reflection of past events, presumably dating from when the lithosphere section considered was located at a plate margin or otherwise involved in extensive major deformation and/or heating. The presence of old mantle lithosphere with largely frozen mineral equilibria will add to and emphasize the compositional heterogeneities within the upper mantle and provide an extensive source of material for contamination of magmas. The apparent absence⁴³ in kimberlites of ultramafic nodules representing the upper part of the upper mantle (at ambient geotherm temperatures <900–1,000 °C) could simply be the result of sampling only frozen mineral equilibria at these depths; with the corollary that ultramafic nodules indicating temperatures of mineral formation around 1,000 °C (± 100 °C?) could be derived from the extensive depth range of mantle lithosphere lying at lower geotherm temperatures. Given the existence of such thicknesses of frozen lithosphere the similarities between nodules from Precambrian and Cretaceous African kimberlites⁴⁴ become less unexpected.

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Mutualism in which ants must be present before plants produce food bodies

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Certain interactions between ants and plants can be classified as mutualisms¹⁻³, with benefit accruing to both members. The plant provides a source of energy, either as solid food or as nectar, and sometimes a domicile such as a hollow stem (or a stem capable of being made hollow by the ants) or hollow stipular thorns. The ants provide the plant with defence against herbivory and/or vine overgrowth. Whether the plant relies entirely or only in part on the ants for its defence, in relationships studied so far it always produces a source of food irrespective of the ants' presence. However, we report here that in the mutualistic relationship between the plant *Piper cenocladum* and the ant *Pheidole bicornis*, the production of food bodies is closely tied to the presence of the ants, so that when they are removed, production of food bodies nearly ceases and when ants reinvade the plant, production begins again. This is the first description of such a relationship in which the food tissue of the plant is induced by the insect.

Risch *et al.*^{2,4} have shown that three Costa Rican species of *Piper* share a mutualistic relationship with *Pheidole bicornis* ants. These *Piper* species are slender, shrub-like plants that commonly grow to a height of several metres. They occur in the understory of mature, wet evergreen forests but are most common in more exposed areas such as light gaps caused by fallen trees or along creeks or paths. The plants provide a domicile within a cavity formed by the appressed, curled margins of the petiole (Fig. 1a), and the ants obtain additional living space by hollowing out the stem. Apparently the growth of the plants is not affected and no important structural damage is done. In return for the domicile and food tissue, the ants seem to help keep the plant free of invading vines, and remove small herbivorous insects and their eggs.

On the recurved adaxial surface of the petiole margins of *P. cenocladum*, single-celled food bodies are produced. They are collected and consumed by the ants. When a food body forms, the nucleus of an epidermal cell enlarges to seven to eight times its normal diameter and the external cell wall extends outwards (Fig. 1b, c). The nucleus then moves into the newly

formed region of the cell and continues to enlarge together with the rest of the cell. The mature food cell (300–500 µm in diameter; Fig. 2) has a dense cytoplasm rich in protein and is filled with lipid droplets. (Chemical analysis by dry weight shows that food cells contain 10.7% protein, 2.1% carbohydrate and 22.2% lipid.)

Our initial observation was made on living plants of *P. cenocladum*, possessing a weak colony of *P. bicornis* ants, which had been established in a greenhouse at Oregon State University. When, after a short period, the colony died, newly produced leaves developed only a few food cells. This condition persisted in all leaves subsequently produced, although the plants thrived in greenhouse conditions. Because there was no precedent for the necessity of ants to be present for food cells to develop, we investigated the relationship between the insect and the plant in experiments conducted in Costa Rica.

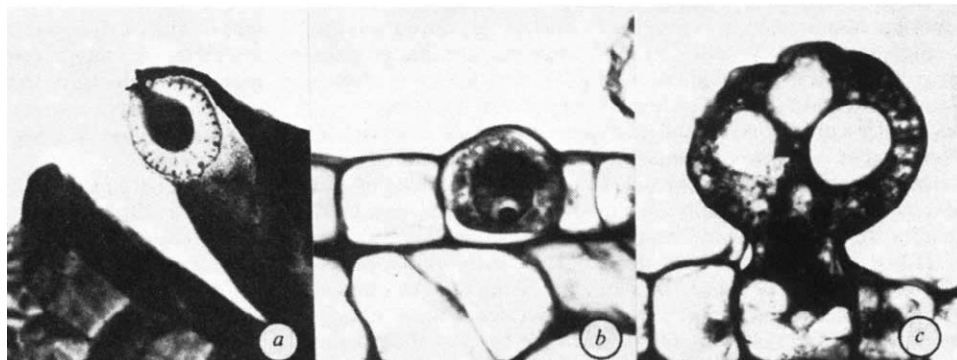
First, we counted the average number of food cells in a *P. cenocladum* petiole tube by dissecting nine petioles from seven plants under a dissecting microscope. The average was 1,500 food cells for a 'typical' petiole. This became the standard against which to compare the results of the ant exclusion experiments described below.

In our first experiment, a 12% aqueous solution of benzene hexachloride (HCH, an insecticide) was injected into the petiole tube of the youngest four leaves of each of nine *P. cenocladum* plants growing at the La Selva Field Station, in the Atlantic lowlands of Costa Rica. After injection, the hole through which ants normally pass from the petioles to the stems was plugged with a sticky resin (Tanglefoot) and the base of the petioles was entirely covered with Tanglefoot to keep ants or other arthropods out of the petioles. After 47 days, all plants were in excellent condition, showing no apparent effects of the HCH. Each treated petiole contained less than 25 healthy food cells, significantly fewer than the number in plants occupied by ants (Wilcoxon signed rank test, $P < 0.01$). There were several large and almost totally decayed food bodies in each of these petioles but virtually no new food bodies were being produced.

In three of the nine plants, active ant colonies were found in petioles below the poisoned area. In those petioles with active ant colonies, the inside of the petiolar sheath was covered with a dense growth of food bodies, similar to that seen in plants not injected. In the remaining six plants, there were no living ants. Although only the top four petioles had received HCH, some of it had probably leaked into the hollow stem and killed the rest of the ant colonies. In these plants, even the petioles below the top four poisoned petioles contained no healthy food bodies.

In our second experiment, ants were added in the field to uncolonized plants of *P. cenocladum* in an isolated population near Turrialba, Costa Rica. By gently prying open the petiolar sheaths, we could see that there were fewer than 50 food bodies in each petiole: we did not actually count them, for that would have destroyed the petioles. *P. bicornis* ant colonies were transplanted to 18 of these plants by attaching an uprooted *P. cenocladum* plant containing an ant colony to each unoccupied plant. (Previous experiments had shown that in these conditions, the colony would usually leave the original plant

Fig. 1 a, Basal portion of a *Piper cenocladum* petiole showing the tube formed by the petiole margins. $\times 2$. b, Early stage in food cell development. Note the expanding outer wall, enlarged nucleus and dense cytoplasm. $\times 820$. c, Mid-stage in food cell development. $\times 820$.



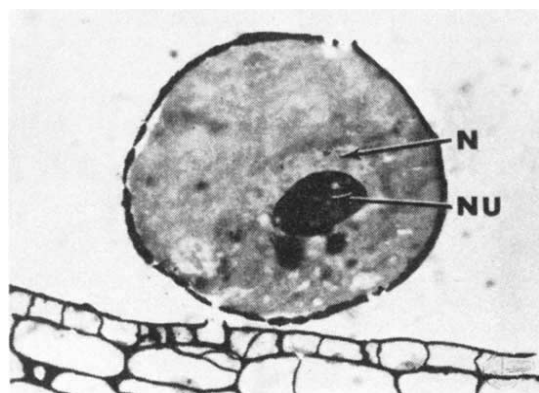


Fig. 2 Mature food body differentiated from a single epidermal cell. N, nucleus; NU, dense nucleolus. $\times 820$.

when it started to wilt and colonize the nearest unoccupied plant.)

After 40 days, 14 of the previously unoccupied plants possessed ant colonies in about one-third of their petioles. Comparison between plants showed that occupied petioles possessed significantly more food cells per petiole ($\bar{x} = 1,020$) than unoccupied petioles ($\bar{x} = 120$) (t -test, $P < 0.01$). In other unoccupied plants of the original population, the average was 19 food bodies per petiole, significantly less than either occupied or unoccupied petioles (t -test, $P < 0.05$).

In our third experiment, occupied *P. cenocladum* plants were transplanted into containers and moved into a greenhouse at Turrialba, Costa Rica. Nine of them wilted and died back, and their ant colonies departed. After a short period, the plants resumed vigorous growth, but produced no food bodies within the petiole tubes. Of those plants which resumed growth, five were given a new *P. bicornis* ant colony, and four were left unoccupied. After 40 days, nondestructive estimation of the petioles showed that the five with ants were producing a full complement of food cells ($> 1,000$ per petiole), whereas the four without ants had almost no food tissues.

In our final experiment, 12 of the potted *P. cenocladum* plants which had retained their ant colony after transplantation were treated with hydrogen cyanide (HCN) gas. This killed the ant colony in 10 plants: 5 were retained in a greenhouse while the other 5 were further isolated in an indoor laboratory. After 53 days, all 10 plants had virtually no food bodies. Four plants were dissected and examined microscopically. Their petioles contained an average of 15 (1–20) food cells (significantly less than 1,500, Wilcoxon signed rank test, $P < 0.01$). No living arthropods, such as mites, were found in any of these dissected plants. Of the six remaining HCN-treated plants, four received transplanted ant colonies and two were set aside as final controls. After 51 days, all remaining plants were dissected. Those possessing an ant colony contained significantly more food cells per petiole ($\bar{x} = 1,811$ per petiole) than the two control plants ($\bar{x} = 22$ per petiole) (t -test, $P < 0.01$).

Each of our four experiments had the same end result: petioles occupied by a colony of *P. bicornis* produced a large complement of food cells ($\sim 1,500$) while the ant-less petioles possessed a food cell population of 5–50. In addition to stimulating food body production, the ants apparently keep their food source free of fungus. No fungal hyphae were ever observed on food bodies in plants with ants. However, in plants without ants (either found naturally in the field or produced by killing off the ant colonies experimentally), the medium- and large-sized food bodies were nearly always covered with fungal hyphae.

It has been assumed that the plants in these relationships derive maximum benefit in immediately being able to support colonization by the protective ants. Therefore, some energy must always be wasted by the plant in the form of food tissue produced before a colony is established. Such food tissue is

either eaten by other animals or falls to the ground. In *P. cenocladum*, however, photosynthate is not used for food tissue before ants arrive. Any energy saved could be put into, for example, the establishment of seedlings or growth. Such energy savings might be especially important to *P. cenocladum* as it grows in the low-light understory of wet, evergreen forests. Three other plants that harbour ants, *Cecropia*, *Acacia* and *Macaranga*, which produce food tissues even in the absence of ants, occur in well lit, disturbed habitats where presumably the amount of photosynthate is not limiting. We have occasionally collected *P. cenocladum* plants containing colonies of *Pheidole* other than *P. bicornis*. There has never been any trace of food bodies in their petioles. Yet in several cases in which *P. bicornis* was found in other petioles of the same plant, those petioles always contained many food bodies. Could it be that the species of *Pheidole* normally occupying *P. cenocladum* is uniquely able to stimulate the production of food bodies in its host?

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Voice pitch as an aid to lipreading

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The totally deafened adult, unable to make use of a hearing aid, has no alternative to lipreading for everyday communication. Lipreading, however, is no substitute for hearing speech. Many lipreaders have great difficulty in ideal conditions and even the best lipreaders find the task demanding and tiring. Prosthetic attempts to substitute for lost hearing have centred on three distinct types of intervention, visual^{1,2}, tactile^{3–6} and electro-cochlear^{7–13}. As none of these is likely to yield a good understanding of speech independent of lipreading in the near future, we have attempted to isolate relatively simple patterns of stimulation that, although not intelligible in themselves, will aid lipreading. From this point of view, the fundamental frequency or 'pitch' of the voice is the most important pattern element because it provides both segmental and suprasegmental information and is practically invisible. It thus complements the visual information already available on the face. As we show here, with the voice pitch presented acoustically, normal listeners can lipread a speaker reading a continuous text at up to two and a half times the rate possible on the basis of lipreading alone. The pitch signal by itself, of course, is completely unintelligible. Although our work is primarily concerned with methods of electrical stimulation of the cochlea¹⁰, it has implications for other sensory substitution techniques, the design of special purpose hearing aids and current theories of speech perception.

Understanding speech solely by vision is difficult for many reasons¹⁴. On the segmental level (pertaining to individual sounds), some articulatory positions are difficult or impossible to see, for example, g and k. Even for those sounds that are visible, few have unique visual cues. So, 'bet' and 'pet' look alike, as do 'vat' and 'fat'. The most important cause of such confusions is that vocal fold activity, although essential for signalling such contrasts, is invisible. The only difference between 'vat' and 'fat'

is that the vocal folds vibrate throughout the *v* and not the *f*. In 'bet' and 'pet', the relevant cue is in the timing of the onset of vocal fold activity relative to the opening of the lips. Not only the presence or absence but also the rate of vocal fold vibration, the pitch contour, is important. This information is spread over two or more segments and is thus said to be suprasegmental. Also invisible, the pitch contour can signal among other things, aspects of syntactic structure, whether a question is being asked or a statement made, and which words are stressed in an utterance. Furthermore, voice pitch is the first component of speech mastered by the developing child¹⁵ and is a major indicator of emotional attitude. Although vocal fold activity is not the only information missing in visible facial gestures, it is probably the major one. We should expect provision of it greatly to improve lipreading ability in those who have acquired an auditory knowledge of speech before becoming deaf.

To compare the relative contribution of various types of extra information to lipreading ability, we have made extensive use of a testing technique known as connected discourse tracking (CDT)³. In CDT, one person, designated the receiver, must repeat back verbatim what another person, the talker, has said. Every word must be correctly repeated; none may be skipped over. The talker decides on the length of segment to speak at one time although the receiver may interrupt. This length varies both between talker-receiver pairs and within pairs since the length of segments increases as the team become more skilful. When the receiver makes an error, the talker has two basic strategies available. First, and most common, he repeats the bit of text that was received in error. Second, he may comment about the error in any number of ways, for example, give a synonym or paraphrase the meaning. Unlike 'shadowing' tasks, never are the talker and receiver speaking at the same time.

Acoustic (but not visual) isolation between talker and receiver is achieved by placing them in two structurally separate adjoining soundproof rooms with thick viewing windows between and masking noise introduced into the receiver's room. This makes the speech of the talker completely inaudible to the receiver. The talker hears the receiver as normal from a microphone mounted on a set of headphones the receiver wears, routed to an amplifier and loudspeaker in the talker's room. As connected speech, rather than isolated segments, is used, the situation faced by the receiver seems close to that faced by the totally deaf in everyday life. This is important, as some aids, although helpful for isolated segments, show no gain for connected speech⁶. To quantify the task, the talker reads from a novel and performance is measured by calculating the average speed of transmission in words per min over the 5-min testing session.

Using this task we have compared lipreading alone with lipreading plus auditory pitch for five different receivers. Under the lipreading alone (LA) condition, receivers heard nothing through the headphones they were wearing and had to rely solely on visual cues. In the voice pitch (VP) condition, they heard the pitch contour of the talker, after suitable processing in order to make the frequency discrimination capabilities of the normal listeners match those shown by electrically stimulated deaf patients. The pitch of the talker was derived through use of a laryngograph which, by measuring impedance changes, senses vocal fold closure using external electrodes on either side of the speaker's neck¹⁶. Each receiver had the same talker for all 24 sessions, making a total of 2 hours per condition per talker-receiver pair. Results as a function of session number are shown in Fig. 1, and the mean rates, averaged over all sessions, are given in Table 1. All receivers are considerably faster with the pitch, although the extent of improvement varies. On the whole, the better lipreaders are helped more by the provision of voice fundamental frequency.

For convenience, we have used the laryngograph for the extraction of voice fundamental frequency, but a practical system must work from the acoustic waveform. Preliminary results of ours show that at least one method of acoustic extraction¹⁷, although not nearly as accurate as a laryngographic technique, gives gains in lipreading ability as large as those

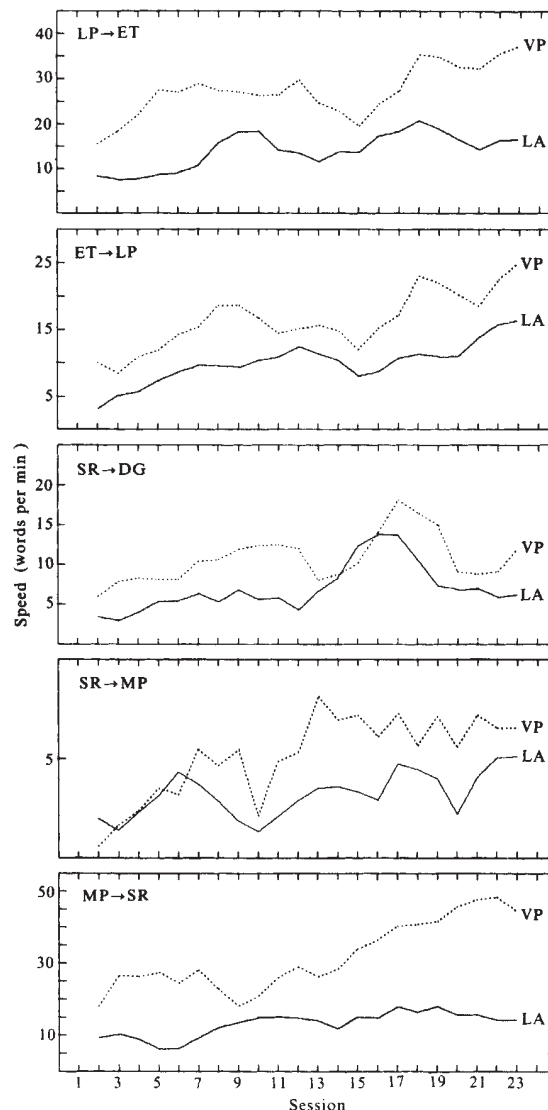


Fig. 1 Speed in words per min as a function of session number for five talker-receiver pairs in a connected discourse tracking task in two conditions. Sessions were 5 min long, making a total of 2 h per condition per talker-receiver pair. These curves result from three-point smoothing of the original data. Note the different scales for the different talker-receiver pairs. In condition LA (lipreading alone), receivers got no auditory information and had to rely totally on lipreading. In condition VP (voice pitch), receivers heard the voice pitch of the talker which was extracted using a laryngograph which senses vocal fold closures by means of a pair of electrodes on either side of the talker's neck. These closures triggered constant-amplitude 20- μ s rectangular pulses. Further processing of this pitch information was necessary as electrically stimulated patients show, on the whole, less acute frequency discrimination capabilities than normal listeners. Although the data are sparse and variable on this issue, the most extensive work²¹ shows that the median percentage differences in frequency needed for discrimination (the so-called difference limens or DLs) are 4, 7.6 and 6.5% at 125, 250 and 500 Hz, respectively. Patients can achieve values as low as 1-4% over this range^{12,21}. We made the normal listening subjects functionally like the deaf by filtering the pulses triggered by the laryngograph low-pass at 20 kHz (12 dB per octave) and high-pass at 4 kHz (48 dB per octave) before presenting them to the receiver through headphones (real ear probe frequency response flat within ± 5 dB from 300 Hz to 8 kHz) at a peak level of 72 dBA (measured on a flat plate coupler). The receiver still hears the pitch contour of the talker, but in a reduced form. DLs measured for such stimuli on nine young and 'intelligent' but naive subjects were 6.8, 4.4 and 2.1% at 75, 150 and 300 Hz. Our simulation is thus quite good at the low frequencies and still reasonable over the range of frequencies adult males and females (the talkers in the study) typically use (70-350 Hz).

Table 1 Results from a connected discourse tracking task for five talker-receiver pairs averaged over 24 5-min sessions

Talker	Receiver	Speed (words per min)		% Gain	Absolute gain (words per min)
		LA	VP		
LP	ET	14.0	27.1	94	13.1
ET	LP	10.0	16.6	66	6.6
SR	DG	6.9	10.7	55	3.8
SR	MP	3.2	5.0	56	1.8
MP	SR	12.9	31.7	146	18.8

Per cent gain is measured by the formula: $100 \times (VP - LA) / LA$ where LA and VP are the speeds attained in each condition.

reported above. Other methods of extraction will certainly prove as adequate.

Given that our simulation is a reasonable approximation to the situation experienced by an electrically stimulated deaf patient, these results augur well for single-channel electro-cochlear stimulation based on the provision of voice pitch¹⁰. Comparisons among different methods, electro-auditory and otherwise, will be difficult until all have been tested by similar means. CDT has been used, however, in testing two tactile aids^{3,6}, giving a percentage gain of the aided over the lipreading alone condition of, at best, 30%. Comparison of such gains with those of Table 1 should give pause to those who maintain that the use of any prosthesis necessitating surgery is premature.

Because pitch, at least when it is presented auditorily, can be of such enormous help in lipreading, it may be fruitful to explore its provision by tactile and visual aids. Initial trials with a single vibrator transmitting pitch information using a temporal code have been disappointing⁵, but better results may be obtained when pitch changes are signalled by changes in the place of stimulation^{18,19}. Also, there are some patients with residual hearing at usable levels who have such severe recruitment (limited dynamic range) that they are unable to use hearing aids. As intensity changes are not necessary for the use of pitch information, such patients may gain much through use of a hearing aid that extracts and presents the pitch alone at the proper intensity.

Finally, these results contribute more generally to our understanding of speech perception. We have isolated one particular speech feature, presented it to subjects in a totally unnatural way, in a frequency region which, in fact, contains little energy during normally-voiced speech. The subjects have, however, been able to analyse this unfamiliar auditory input with reference to their existing knowledge of speech patterns and combine it effectively with visual information. The possibility that a single pattern feature can be used in this way is a necessary consequence of the point of view that speech perception depends primarily on separable auditory features which are extracted by the listener from the complex acoustic speech stream²⁰.

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Magnetic sense of direction in woodmice for route-based navigation

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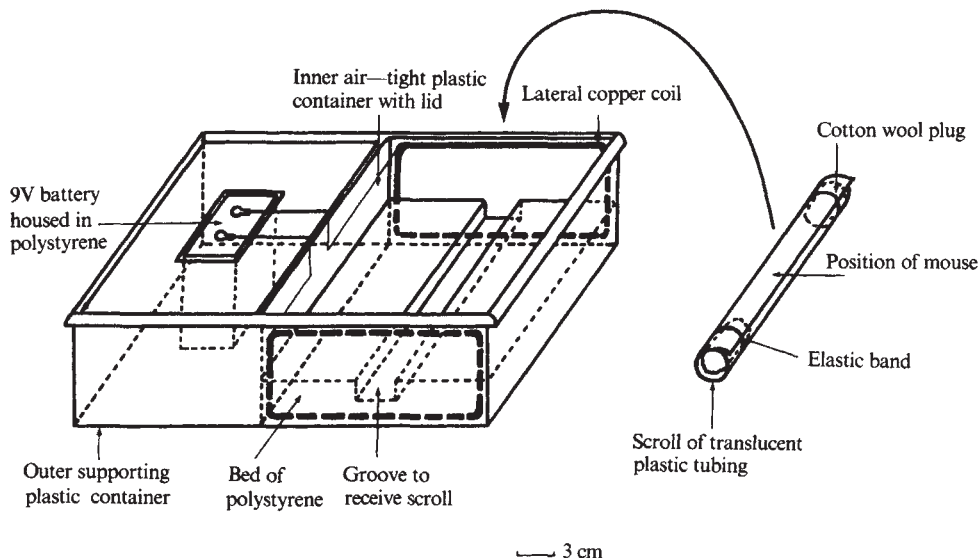
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Orientation to the Earth's magnetic field has been shown for bacteria, planarians, molluscs, insects, elasmobranch fish, salamanders and birds^{1,2}. Recent work indicates that humans may also have a magnetic sense of direction^{3,4}. We present here the first evidence for such a sense in a mammal other than man, the European woodmouse. In addition, by manipulation of the magnetic information available during the outward journey (an approach used previously with pigeons^{5,6}), we present the first clear evidence that both route-based and location-based mechanisms can be involved in goal orientation following displacement.

All experiments were conducted on the European woodmouse, *Apodemus sylvaticus*, between 10.00 and 18.00 GMT from 23 June to 23 July 1980, at Woodchester Park Field Centre, Gloucestershire, UK. After capture in a Longworth trap, each individually fur-clipped mouse was allowed to enter an adjustable translucent plastic scroll which was then placed inside a transportation cage (Fig. 1). This cage was air tight and light proof, and supported two lateral coils of copper wire which could be activated to manipulate the ambient magnetic field. After a 2-min adaptation period, the cage plus animal was carried in a straight line roughly 40 m due north to a test site, the animals spending approximately 4 min inside the transportation cage. At this site the induced field was deactivated before the transportation cage was uncovered, taking care to ensure that the position of the Sun could not be detected by the mouse during the period from activation to deactivation of the artificial magnetic field. At the test site, the tube containing the animal was removed from the transportation cage and the mouse allowed to enter an orientation cage, which was housed inside a large cardboard box (70 cm diameter and 60 cm high). This box restricted both visual and olfactory cues, the latter being reduced further by the addition of Perspex doors to the orientation cage. The cage was positioned so that its four arms were directed towards magnetic north, south, east, and west. Four guy ropes anchored the cardboard screen firmly to the ground. The method was as described by Mather and Baker⁷. The cardboard box lid was folded over to create a 25-cm diameter window above the orientation cage. Consequently, only the sky directly overhead could be seen by the mouse from within the cage, any features on the horizon being screened by the box. During roughly half of the tests the sun shone into the box, though the Sun's disk could rarely be seen directly by the mouse in the cage.

Testing consisted of recording the time spent in each of the four arms of the orientation cage during a 4-min test period, observation being through small peep holes in the cardboard

Fig. 1 The transportation cage consisted of a translucent air-tight plastic container (18×18×9 cm), the inside of which supported lateral coils made from 100 turns of 20 standard wire gauge insulated copper wire, and powered by a 9 V battery. An 82-Ω resistor in the circuit resulted in a current of 65 mA, producing a magnetic field of ~0.37 G between the coils. When this field was directly opposed to the horizontal component of the Earth's normal magnetic field, a near mirror image of the local geomagnetic field was produced. The vertical field at the animal's position was changed by less than 5% whereas the horizontal field was reversed and brought to within 115% of the natural value. This gave a total field strength of 0.52 G. As a result, the magnetic field through the experimental animal could be adjusted so that when the cage was orientated along the north-south axis (as it was during experimental displacement of the test animals), the horizontal component of the ambient magnetic field could be shifted by 180°. Alternatively, the battery could be disconnected so that an animal in the centre of the deactivated transportation cage would experience the Earth's unchanged magnetic field, and could therefore serve as a control. The animal was retained in a plastic scroll, the diameter of which could be adjusted by loosening two supporting elastic bands. This ensured both that the mouse remained in the same orientation (facing direction of travel) during transport to the test site, and that the tube was loose enough for the animal to suffer no ill effects. A cotton wool plug at either end retained the animal in the central part of the scroll, which was then placed in a groove in a bed of polystyrene inside the transportation cage.



box. (We have shown previously that observer position has no significant effect on the orientation response of rodents in this design of experiment⁷. In fact, the present investigation had a built-in control in that as long as observer position remained constant following both control and experimental displacements, any influence of observer position could not account for differences in the orientation of the two groups. Nevertheless, the observers' location was always neutral (at 90°) to the home direction and changed from east to west half-way through the experimental series.) After each test, the orientation cage was covered by a black polythene bag and used to return the animal to its point of capture.

Twenty different animals were tested (13 males), no animal being used on two consecutive days⁷. The first series of experiments consisted of 33 separate displacement occasions, 16 of which used a reversed magnetic field (Fig. 1) during the outward journey. Another 17 displacements served as controls, the coils being disconnected during displacement, the animals thereby experiencing the Earth's normal magnetic field. The influence of smell of previous occupant was controlled by maintaining the orientation cage in the same physical orientation (the same arm pointing north) during testing, and arranging the tests in a sequence of two 'activated' displacements followed by two controls.

Statistical analysis was carried out in two stages, as described by Batschelet⁸⁻¹⁰. Time spent in each of the four arms of the

orientation cage was used in a first-order analysis to calculate the mean vector (r, θ) for each 4-min test, where r is the length and θ the mean angle. The mean angles were then subjected to second-order analysis¹⁰. Non-uniformity was examined by Rayleigh's Z -test⁸, and clustering around a predicted direction by the V -test⁹. The Watson $U^2_{n,m}$ non-parametric two-sample test (test statistic U^2)⁸ was also used. Statistics are presented with home direction as 0°.

The results (see Table 1 and Fig. 2) show that there is a marked dichotomy in the directional preferences of the control and experimental animals, the distributions of direction for the two groups being significantly different ($U^2_{17,16} = 0.402$; $P < 0.001$). After transportation involving minimal exposure to visual and olfactory cues, but in the presence of the Earth's normal magnetic field, the mice showed homeward orientation (V -test, Table 1) despite the continuing restriction of these cues by the screen and perspex doors at the test site. When the magnetic vector was deflected by reversing the horizontal component of the magnetic field during displacement, subsequent orientation by the mice at the test site was shifted away from home by 131°, resulting in a 117° difference in the mean orientation of the two groups. Experimentals are significantly clustered about 180° from home ($u = 1.876$, $P < 0.05$), but are not quite significantly clustered about 180° from the mean angle for controls ($u = 1.298$, $P < 0.10$).

It has been suggested that the ability of animals to detect a magnetic field may be associated with deposits of magnetite which have been found in a variety of organisms². In vertebrates, magnetic remanence has been found most often in the head region. We have undertaken pilot investigations into the possibility that similar deposits may exist in the rodent head.

Three species of rodents were used, the European woodmouse, *A. sylvaticus*, the bank vole, *Clethrionomys glareolus*, and the house mouse, *Mus musculus*, giving a total of 12 animals which had been deep frozen for up to 3 months before use. Each specimen was then embedded in a small block of deionized ice and retained in a plastic container for insertion into a par-a-static magnetometer. The heads were tested whole for natural remanence (NRM, a net field due to a preferential alignment among individual magnets), and inducible remanence (IRM, a measure of the total amount of magnetic material). Immediately before testing for IRM, the specimens were exposed to a

Table 1 Orientation performance in the test apparatus; summary of second-order analysis

Variable	No. of tests	Mean angle (θ)	Mean vector (r)	Z	u (versus home 0°)
Normal field plus screen	17	+14°	0.416	2.940	2.353*
Reversed field plus screen	16	+131°	0.505	4.088†	-1.876
Reversed field no screen	20	+7°	0.483	4.662*	3.031‡

Z = Rayleigh's test, u = V -test.

* $P < 0.01$; † $P < 0.05$; ‡ $P < 0.005$.

2,000-G field to align any permanently magnetic material. The heads were subsequently dissected, glass instruments being used to prevent possible contamination from metallic tools, and the various parts were retested for IRM (see Table 2).

All samples exhibited some NRM and even greater IRM, which was strongest in the anterior part of the head in an area associated roughly with the olfactory region but not directly with brain tissue. The level of magnetic remanence found in rodent heads is comparable with that found in pigeons (10^{-6} – 10^{-5} e.m.u.)¹¹ and bees (10^{-6} e.m.u.)¹², but less than that found in the dolphin (10^{-5} e.m.u.)¹³.

The fact that mice, which had experienced a reversed magnetic field during displacement and been allowed no visual or olfactory cues en route and only limited olfactory cues at the destination, orientated away from home suggests that route-based navigation (estimating home direction by monitoring the outward journey)⁴ was taking place even in the absence of visual cues, and the involvement of a magnetic sense of direction in this ability. Alternatively, there could be short-term after-effects of exposure to altered magnetic fields. If so, fields experienced during transportation might affect subsequent navigation at the test site. However, we consider this interpretation improbable, particularly as the natural and artificial field strengths were so similar that it seems unlikely for the two to have differing effects.

Having found that we could disrupt the initial orientation response, by restoring visual and olfactory cues at the destination, a study of the involvement of these cues in location-based navigation (perceiving cues at the release site and from these determining home direction)⁴ was possible. After the first series of experiments had been conducted, a second series was carried out in which the magnetic field was reversed during displacement, as before, but no screen (cardboard box) was placed around the orientation cage at the test site. All other procedures were identical except that the observers were sited 5 m away. In all, 20 different individuals (15 males) were tested once each (5 had been used in the first series of experiments), over a period of 6 days.

The results are presented in Fig. 2c and show that when animals experience a reversed magnetic field during displacement but are allowed relatively unrestricted access to visual cues at the test site, they orient not far away from home, as in Fig. 2b, but towards home (Fig. 2c). Comparison of the first-order mean angles of the two groups, which both experienced a reversed magnetic field during the outward journey, shows a significant difference ($U^2_{20,16} = 0.273$, $P < 0.01$), whereas the preferred directions of those animals experiencing a normal field plus screen and a reversed field but no screen are not significantly different ($U^2_{20,17} = 0.063$, $P > 0.1$).

Table 2 Summary of natural remanence (NRM) and inducible remanence (IRM) in various regions of the rodent head

		<i>n</i>	Mean magnetic moments	Range
			($\times 10^{-6}$ e.m.u. g ⁻¹)	
Container				
Empty	NRM	4	0.63	0.28–1.05
Plus ice	NRM	4	0.83	0.59–1.23
	IRM	4	0.96	0.51–1.45
Whole head	NRM	11	1.33	0.29–7.81
	IRM	12	6.31	1.14–18.31
Head (IRM)				
	Cranium	3	3.08	1.20–5.57
	Lower jaw	7	0.75	0.43–1.64
Cranium (IRM)				
	Anterior	6	5.13	1.26–16.13
	Posterior	9	1.23	0.59–2.55
Anterior cranium (IRM)				
	Anterior	5	1.93	0.47–4.22
	Posterior	5	0.82	0.27–2.01
	Dorsal	6	1.92	0.71–4.01
	Ventral	6	1.31	0.71–2.99

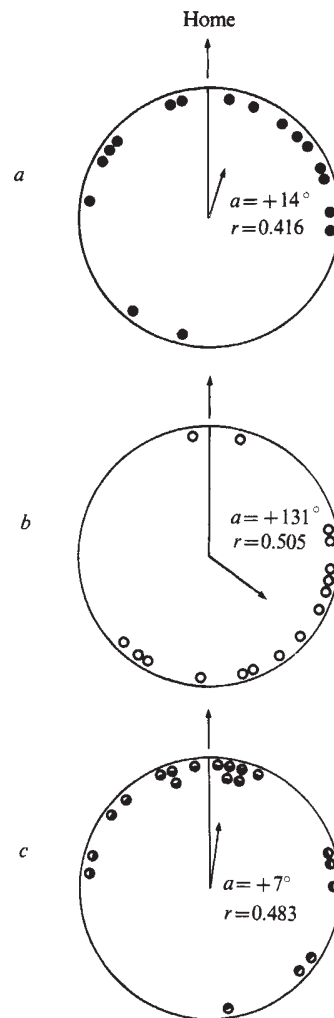


Fig. 2 Effect of conditions during displacement and at the release site on the goal orientation of woodmice. Each dot represents the mean angle (θ , first-order analysis) for an individual during a 4 min test period. Arrows within the circles show the second-order mean vectors of the estimates. Statistics show the mean angle (a°) and the length (r) of the mean vector. Angles are expressed with home direction 0° . a, Normal field plus screen; b, reversed field plus screen; c, reversed field, no screen.

Our experiments have shown that: (1) woodmice do have a magnetic sense of direction; (2) they can sample the magnetic field during experimental displacement and can use this information for route-based navigation; and (3) the magnetic information collected during the displacement journey may become subservient to other cues obtained at the test site if the information from the two sources conflict. We stress, therefore, the importance when studying route-based navigation of restricting cues available at the test site, and when investigating location-based navigation, of manipulating in a predictable way the animal's ability to monitor its outward journey.

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Stimulation of aldosterone production by β -melanotropin

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Experimental and clinical evidence suggests that a non-ACTH pituitary factor(s) is important in the regulation of aldosterone secretion in certain conditions^{1,2}. Earlier studies have suggested that β -melanotropin (β -MSH) has only weak steroidogenic potency on the adrenal gland in the rat^{3,4}, but recently Challis *et al.*⁵ and Rudman *et al.*⁶ have reported that α - and β -MSH have a trophic action on fetal adrenal gland. However, little is known about the effect of β -MSH on aldosterone production. We recently reported that β -lipotropin (β -LPH) stimulates aldosterone production in rat capsular cells⁷. As the amino acid sequence of β -MSH is contained within the β -LPH structure^{8,9}, we have now investigated the aldosterone-stimulating activity of synthetic β -MSH. Our results indicate that β -MSH causes a dose-dependent increase in aldosterone production in rat capsular cells which is blocked by a synthetic β -MSH analogue. The synthetic hormone does not increase corticosterone production in decapsular cells.

Collagenase-dispersed capsular (mainly zona glomerulosa cells) and decapsular (zona fasciculata and reticularis cells) adrenal cells from 20 female Sprague-Dawley rats on normal sodium diets were prepared for each experiment according to previously described methods¹⁰. In this capsular cell dispersion technique the degree of contamination with zona fasciculata cells has been reported to be less than 10% (ref. 11). Camel β -melanotropin (β_{Cl} -MSH) and the analogue, [Gly¹⁰]- β_{Cl} -MSH, and ovine β -LPH were synthetic products as described previously^{12–14}.

As shown in Fig. 1a, 10^{-10} M [Asp¹, Ile⁵]-angiotensin II (AII, Sigma) and ACTH_{1–24} (Ciba) caused a significant increase in aldosterone production, maximal with 10^{-8} M AII or ACTH_{1–24}. Also, a significant increase in aldosterone was produced by 10^{-8} M β -MSH (from the control value 24.9 ± 3.6 (s.e.) ng per 100,000 capsular cells to 45.2 ± 3.9 ; $P < 0.01$) and maximum increase was obtained with 10^{-6} M β -MSH. The maximum aldosterone response obtained with β -MSH, however, is about the same as with AII. Figure 1b shows that ACTH_{1–24} has a potent stimulating effect on corticosterone production by decapsular cells, whereas β -MSH has no effect. The effect of the β -MSH analogue on the response of capsular cells to half-maximal aldosterone-stimulating doses of β -MSH and β -LPH (3×10^{-8} M), and of ACTH_{1–24} and AII (1.5×10^{-10} M) are shown in Fig. 2. The analogue caused ~30% inhibition of aldosterone production induced by β -MSH at a molar ratio of 100:1, and ~80% inhibition at a molar ratio of

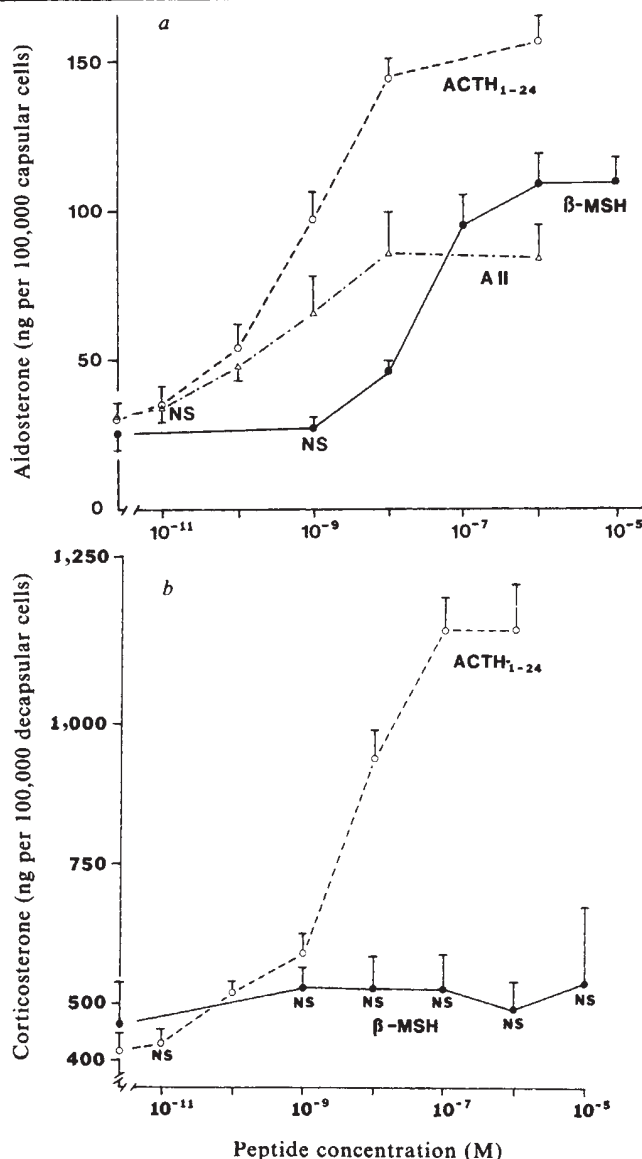


Fig. 1 a, Aldosterone production by AII, ACTH_{1–24} and β -MSH in rat adrenal capsular cells. b, Corticosterone production by ACTH_{1–24} and β -MSH in rat adrenal decapsular cells. Each point indicates mean $\pm$ s.e. from three different experiments. After decapsulation, adrenal glands were removed and separated into capsular and decapsular portions. In a, capsular portions were minced into small pieces and incubated with collagenase (2 mg ml^{-1}) for 30 min. Collagenase-treated capsular cells were incubated in 1 ml medium 199 containing 2 mg ml^{-1} of bovine serum albumin in duplicate with various amounts of AII, ACTH_{1–24} and β -MSH at 37°C for 2 h. In b, collagenase-treated decapsular cells were incubated in duplicate with various amounts of ACTH_{1–24} and β -MSH at 37°C for 2 h. AII did not stimulate corticosterone production in decapsular cells (data not shown). NS: not significantly different from the control value; all other points significantly different from the control value ($P < 0.01$).

1,000:1, and inhibited the β -LPH-induced aldosterone production to a similar extent at the same molar ratios. However, it did not affect ACTH_{1–24} or AII-induced aldosterone production at molar ratios from 10:1 to 10,000:1. In one experiment we found that a 30,000 molar excess of β -MSH analogue did not affect ACTH-induced aldosterone production, and in another, that the β -MSH analogue itself has no effect on aldosterone production at doses from 10^{-9} M to 3×10^{-5} M.

Studies by Page *et al.*¹⁵ showed that α -MSH plus growth hormone stimulated aldosterone production in hypophysectomized sodium depleted rats. More recently, Vinson *et al.*¹⁶

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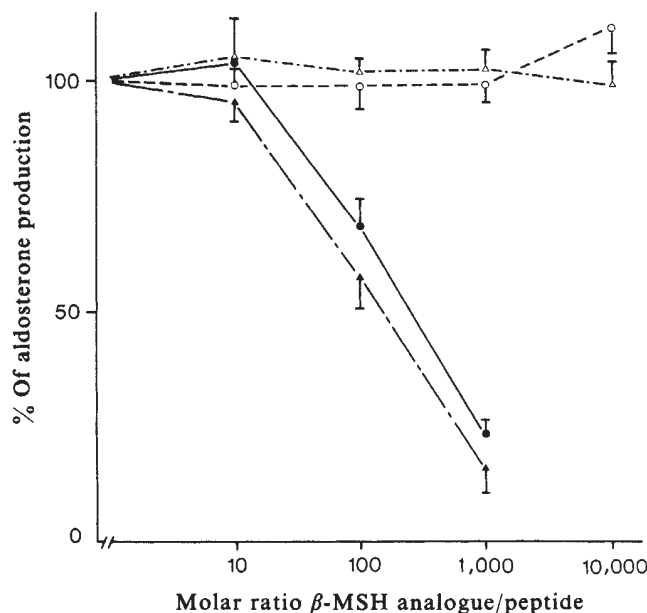


Fig. 2 Inhibition of aldosterone responses to half-maximal stimulating doses of β -MSH and β -LPH (3×10^{-8} M), and ACTH₁₋₂₄ and AII (1.5×10^{-10} M) by varying concentrations of β -MSH analogue. Collagenase-treated capsular cell suspensions were preincubated with various amounts (1.5×10^{-9} M to 3×10^{-5} M) of β -MSH analogue for 5 min and cell suspensions incubated with 3×10^{-8} M β -MSH or β -LPH, and with 1.5×10^{-10} M ACTH₁₋₂₄ or AII for 2 h. Each point shows mean $\pm$ s.e. from three different experiments. $\bullet$, β -MSH; $\blacktriangle$, β -LPH; $\circ$, ACTH₁₋₂₄; $\triangle$, AII.

reported that α -MSH has an adrenal zona glomerulosa-stimulating effect in the rat. The present data show that β -MSH has an aldosterone-stimulating effect on rat zona glomerulosa cells (Fig. 1a) and does not stimulate corticosterone production in rat adrenal decapsular cells (Fig. 1b). Although the concentration of β -MSH needed for half-maximal stimulation of aldosterone production was ~ 100 times greater than that of ACTH₁₋₂₄, we compared synthetic camel β -MSH with ACTH₁₋₂₄, which is known to be the biologically active sequence common to all species. As Lohmar *et al.*¹⁷ and Lis *et al.*¹⁸ reported some species differences in the lipolytic activity of β -LPH, it is possible that there are species differences in the aldosterone response to β -MSH. Moreover, recent evidence suggests that in humans β -MSH is an artefact due to degradation of β -LPH during extraction^{19,20}. The physiological significance of β -MSH in the regulation of aldosterone secretion will therefore clearly require further study.

Because earlier studies showed that β -endorphin (β -LPH₆₁₋₉₁) has no steroidogenic properties⁷, it seemed logical that γ -LPH (β -LPH₁₋₅₈) was the aldosterone-stimulating segment of β -LPH, and we have found that β -MSH (β -LPH₄₁₋₅₈), the carboxy-terminal segment of γ -LPH, contained aldosterone-stimulating activity. The demonstration that the β -MSH analogue inhibited both β -MSH and β -LPH activity to a similar extent suggests that the active core of β -LPH is β -MSH. Furthermore, the finding that the analogue inhibits β -LPH action but not ACTH₁₋₂₄ or AII actions suggests that the zona glomerulosa receptor for β -LPH or β -MSH may be different from the ACTH and AII receptors.

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Circulating human pituitary pro- γ -melanotropin enhances the adrenal response to ACTH

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The amino-terminal region of the common corticotropin/ β -lipotropin (β -LPH) precursor has been identified in the AtT-20 mouse tumour cell as a glycopeptide with an apparent molecular weight of 16,000 (the '16K fragment')^{1,2}. A third melanotropin core sequence or γ -MSH similar to that found in ACTH and β -LPH was predicted to occur in this glycopeptide from the complementary DNA sequence of mRNA isolated from bovine pituitary intermediate tissue³. Recently, the mouse 16K fragment has been found to have a small but significant potentiation on the corticosteroidogenesis elicited by ACTH in a static cell system, an effect that could be enhanced when the glycopeptide was pretreated with trypsin⁴. This synergism could also be mimicked by synthetic γ -MSH peptides *in vitro* and *in vivo*⁵. We report here the potentiating properties of a naturally occurring human pro- γ -MSH glycopeptide⁶ on the ACTH-induced steroidogenic response of isolated perfused rat and human adrenocortical cells.

The *in vitro* superfusion studies of isolated adrenal cells used a modification of the system reported by Lowry and McMartin⁷. Female Wistar rats (200-220 g) were decapitated and the adrenal glands removed. The capsular tissue (namely zona glomerulosa cells) or the decapsulated tissue (zona fasciculata-reticularis cells) was then treated as previously described^{8,9}. Two perfusion cell columns were prepared for each experiment; one column was always used as a control. The cells were checked for viability and counted before suspending in Bio-Gel. Each column contained $\sim 1 \times 10^6$ cells. Using this technique the fasciculata-reticularis contamination of the glomerulosa preparation was 5-10% (ref. 10) as assessed by light microscopy and a Coulter counting system. For the preparation of isolated human adrenal cells, adrenocortical tissue obtained at total nephrectomy for renal carcinoma was treated in the same manner as described for rat adrenal tissue.

The cells were stimulated with ACTH₁₋₂₄ 1-5 ng l⁻¹, ACTH₁₋₃₉ 2-10 ng l⁻¹, Pro- γ -MSH₁₋₇₇ (ref. 6) (γ -MSH precursor Trp 105 to Gln 29, numbering system of Nakanishi *et al.*³) 8×10^{-11} - 5×10^{-9} mol l⁻¹ (10-min pulses, 30-40 min between each pulse) alone or during γ -MSH precursor infusion and fractions were collected every 5 min. The concentrations of γ -MSH precursor used in these studies were based on the plasma levels of this polypeptide measured by radioimmunoassay¹¹. The molecular size of the immunoreactive γ -MSH

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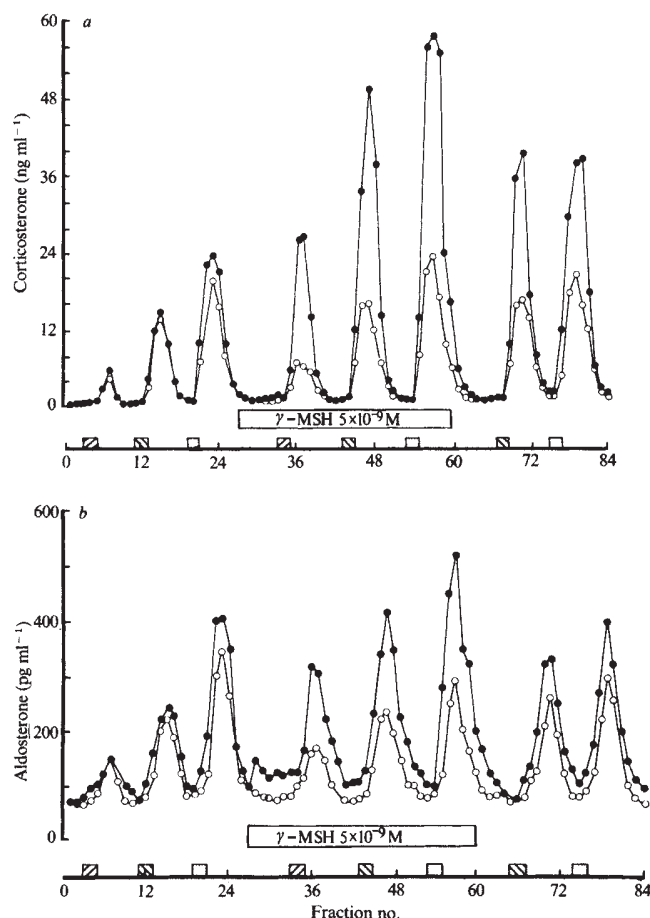


Fig. 1 Corticosterone (a) and aldosterone (b) secretion by isolated perfused rat zona glomerulosa cells in response to pulses of ACTH₁₋₂₄ given before, during and after an infusion of γ -MSH precursor (5×10^{-9} mol l⁻¹). The intact molecule of the precursor (77 amino acids) was found to circulate in the plasma of normal subjects and patients with ectopic ACTH syndrome, Nelson's syndrome and pituitary-dependent Cushing's disease as checked by affinity column chromatography. ACTH₁₋₂₄ and γ -MSH precursor were dissolved in 0.9% sodium chloride solution containing 10 mM HCl, 1 g l⁻¹ bovine serum albumin, 1 g l⁻¹ polypeptide (Sigma) and 100 mg l⁻¹ lima bean trypsin inhibitor. Responses in the column infused with γ -MSH precursor (●) are compared with those in a control column (○). Doses of ACTH₁₋₂₄ given were 1 ng l⁻¹ (□), 2.5 ng l⁻¹ (▨) and 5 ng l⁻¹ (▩). γ -MSH precursor was infused in the experimental columns only between fractions 27–60.

polypeptide in plasma corresponds to a 77-amino acid glycopeptide¹¹. Aldosterone levels were measured in the superfusates by the specific, direct radioimmunoassay developed for human plasma¹², which as we have since shown^{8,13} gives results comparable to those of methods which involve prior extraction of the aldosterone. Corticosterone was measured in the superfusates by a highly specific, direct radioimmunoassay¹⁴, which has also been checked against techniques involving extraction¹⁴. The corticosterone assay uses ¹²⁵I-corticosterone-iodo-histamine as ligand and highly specific corticosterone antiserum raised in rabbits against corticosterone-3-mono-oxime-bovine serum albumin immunogen. The specificity of the antibody was confirmed by the negligible cross-reactivities of this antiserum with potential interfering steroids.

ACTH₁₋₂₄ produced a dose-dependent increase in corticosterone and aldosterone production by rat zona glomerulosa cells (Fig. 1). When pro- γ -MSH₁₋₇₇ was infused in one column there was a significant potentiation of the corticosterone response to ACTH₁₋₂₄ which persisted even after the γ -MSH precursor infusion was stopped (Fig. 1a). The aldosterone response to

ACTH was also potentiated by γ -MSH precursor but this potentiation was less marked than that observed for corticosterone (Fig. 1b). Similar results were obtained when rat zona glomerulosa cells were stimulated by ACTH₁₋₃₉ during γ -MSH precursor infusion. These findings were reproducible and Fig. 2 shows the responses of aldosterone and corticosterone produced by ACTH₁₋₂₄ and ACTH₁₋₃₉ using isolated superfused rat zona glomerulosa cells with or without the infusion of γ -MSH precursor (5×10^{-9} mol l⁻¹). There was a significant three- to fivefold increase ($P < 0.001$) in corticosterone production and 0.75-fold ($P < 0.01$) in aldosterone production by ACTH₁₋₂₄ and γ -MSH precursor infusion compared with ACTH₁₋₂₄ alone. ACTH₁₋₃₉ also produced a significant potentiation in corticosterone (1.5–2.5-fold, $P < 0.001$) and aldosterone (1.0–2.0-fold, $P < 0.001$) production during γ -MSH precursor infusion.

ACTH₁₋₂₄ and ACTH₁₋₃₉ produced a dose-dependent increase in corticosterone production by rat zona fasciculata-reticularis cells (Fig. 3a) which was also potentiated by continuous infusion of γ -MSH precursor, as can be seen by comparing the results in the experimental column with those produced by ACTH alone in the control column (Fig. 3a).

Pro- γ -MSH₁₋₇₇ alone (8×10^{-11} – 4×10^{-9} mol l⁻¹) did not significantly increase steroidogenesis in either zona glomerulosa or zona fasciculata-reticularis cells, although a slight effect on aldosterone production was observed at the beginning of a continuous infusion (Fig. 1). Also, the precursor did not significantly potentiate the ACTH₁₋₂₄ response of cells of either tissue when given simultaneously with ACTH in bolus doses (as confirmed by Kruskal-Wallis one-way analysis of variance by ranks). The effect of an ACTH and γ -MSH precursor bolus dose on steroidogenesis was potentiated (Fig. 3b) following a 30-min priming dose of γ -MSH precursor (4×10^{-9} mol l⁻¹), and this effect could be maintained for some time by simultaneous bolus doses of the glycopeptide with ACTH. Isolated perfused human adrenal cells containing a mixture of zona glomerulosa and

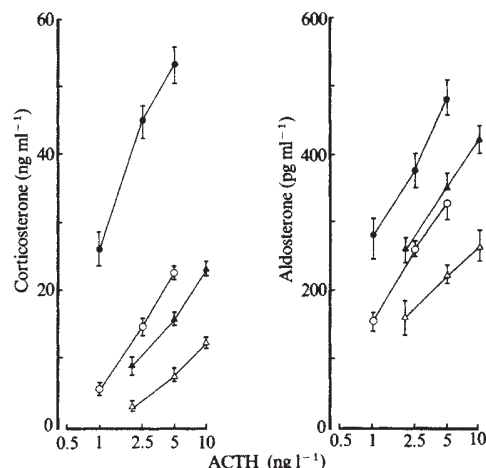


Fig. 2 A summary of the responses of aldosterone and corticosterone produced by ACTH₁₋₂₄ (1.0 ng l⁻¹, $n = 5$; 2.5 ng l⁻¹, $n = 5$; 5.0 ng l⁻¹, $n = 6$) and ACTH₁₋₃₉ (2.0 ng l⁻¹, $n = 6$; 5.0 ng l⁻¹, $n = 4$; 10.0 ng l⁻¹, $n = 5$) using isolated superfused rat zona glomerulosa cells with (●, ACTH₁₋₂₄; ▲, ACTH₁₋₃₉) or without (○, ACTH₁₋₂₄; △, ACTH₁₋₃₉) the infusion of pro- γ -MSH (5×10^{-9} mol l⁻¹). For corticosterone there was a three- to fivefold increase after ACTH₁₋₂₄ and pro- γ -MSH infusion compared with ACTH as bolus doses alone, which was significant ($P < 0.001$) for all doses of ACTH₁₋₂₄. For aldosterone there was a 0.5–1.0-fold increase of the ACTH₁₋₂₄ and pro- γ -MSH infusion compared with ACTH alone. This potentiation was also significant ($P < 0.01$ for ACTH 1.0 ng l⁻¹; $P < 0.005$ for ACTH 2.5 ng l⁻¹; $P < 0.001$ for ACTH 5.0 ng l⁻¹). For ACTH₁₋₃₉ the potentiation in corticosterone was 1.5–2.5-fold ($P < 0.001$) and aldosterone 1.0–2.0-fold ($P < 0.001$) during pro- γ -MSH infusion. Results are the mean $\pm$ s.e.m.

fasciculata-reticularis cells behaved similarly to rat adrenal cells, producing an increase in corticosterone (twofold) and aldosterone (20–40%) secretion to bolus doses of ACTH during an infusion of pro- γ -MSH (Table 1).

Thus, the intact pro- γ -MSH_{1–77} can potentiate the action of ACTH_{1–24} or ACTH_{1–39} on isolated superfused rat and human zona glomerulosa and zona fasciculata-reticularis cells to secrete corticosterone and aldosterone. This action was only apparent when the cells were exposed to a priming infusion or to a

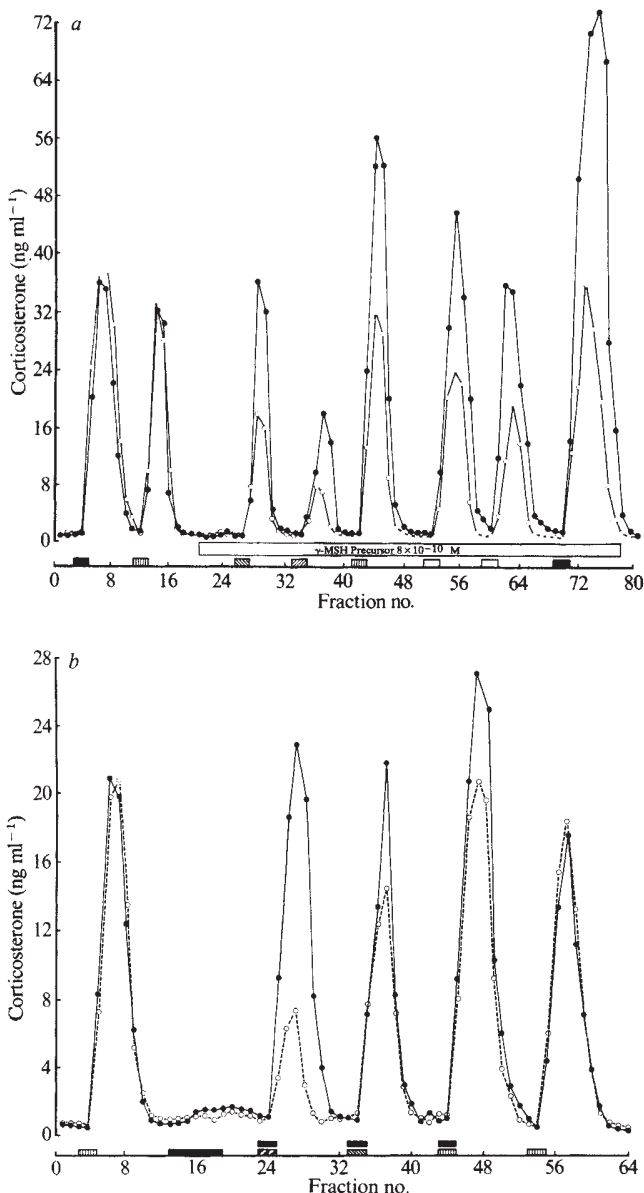


Fig. 3 *a*, Corticosterone secretion by isolated perfused rat zona fasciculata-reticularis cells in response to pulses of ACTH_{1–24} and ACTH_{1–39} given before, during and after an infusion of pro- γ -MSH (8 × 10⁻¹⁰ mol l⁻¹). Responses in the column infused with γ -MSH precursor (●) are compared with those in a control column (○). Doses of ACTH_{1–24} given were 1 ng l⁻¹ (□), 2.5 ng l⁻¹ (▨) and 5 ng l⁻¹ (■), and of ACTH_{1–39}, 2 ng l⁻¹ (▤), 5 ng l⁻¹ (▥) and 10 ng l⁻¹ (▧). γ -MSH precursor was infused in the experimental column only between fractions 20–78. *b*, Corticosterone responses by isolated perfused rat zona glomerulosa cells in response to pulses of ACTH_{1–24} (▨ 1 ng l⁻¹; ▤ 2.5 ng l⁻¹; ▥ 5 ng l⁻¹) given either alone in control column (○) or together with γ -MSH precursor (▨, 4 × 10⁻⁹ mol l⁻¹) (●) after a 30-min priming infusion of γ -MSH precursor (▨, fractions 13–19). The first and last bolus doses of ACTH in the experimental column were given without γ -MSH precursor.

Table 1 Aldosterone and corticosterone secretion by isolated perfused human adrenocortical cells in response to pulses of ACTH_{1–24} (1–5 ng l⁻¹) given before and during an infusion of γ -MSH_{1–77}

Secretagogue	Aldosterone (pg ml ⁻¹ , mean ± s.d.)	Corticosterone (ng ml ⁻¹ , mean ± s.d.)
ACTH	Basal value	69.8 ± 9.0 (8)
	ACTH (1 ng l ⁻¹)	0.5 ± 0.15 (8)
	ACTH (2.5 ng l ⁻¹)	2.62 ± 0.45 (5)
	ACTH (5 ng l ⁻¹)	3.28 ± 0.62 (8)
	ACTH (5 ng l ⁻¹)	3.95 ± 0.92 (5)
ACTH + pro- γ -MSH (4 × 10 ⁻⁹ M)	Basal value	74.6 ± 11.6 (8)
	ACTH (1 ng l ⁻¹)	0.55 ± 0.06 (8)
	ACTH (2.5 ng l ⁻¹)	4.28 ± 0.83* (4)
	ACTH (2.5 ng l ⁻¹)	5.74 ± 1.66* (4)
	ACTH (5 ng l ⁻¹)	8.15 ± 2.98* (4)

* There was a significant potentiation ($P < 0.01$) in the response to ACTH during γ -MSH precursor infusion as confirmed by Mann-Whitney U -test. Values in parentheses represent the number of experiments.

simultaneous continuous infusion and was more marked on corticosterone than on aldosterone production. It therefore seems that the potentiation of ACTH-induced steroidogenesis by the naturally occurring intact form of pro- γ -MSH is time dependent. As there would be little or no degradation of pro- γ -MSH by contaminating peptidases in the superfused system used in our study, the physiological relationship to observations made with trypsin-treated 16K fragment⁴ and synthetic γ -MSH⁵ is unclear.

We cannot altogether rule out the possibility that the potentiation of aldosterone production by zona glomerulosa cells is due to contaminating fasciculata cells providing the precursor corticosterone. This does occur in static systems but is less likely to occur in superfusion systems. Furthermore, the fact that ACTH and serotonin induce aldosterone secretion by our zona glomerulosa cells to a similar extent suggests that fasciculata contamination is not a major problem. We believe instead that the potentiation is related only to the effects of ACTH, a view confirmed by the lack of potentiating action of γ -MSH precursor on other zona glomerulosa stimulators such as angiotensin II, potassium or serotonin.

Our results suggest that the potentiating action of γ -MSH precursor may play an important part in modulating ACTH-induced steroidogenesis. A specific effect of two products of ACTH biosynthesis [α -MSH (ref. 15) and β -LPH (ref. 16)] on aldosterone production by zona glomerulosa cells has recently been observed and Pedersen *et al.*⁵ have reported a direct effect of synthetic γ -MSH on adrenal cholesterol ester hydrolase activity. However, because, in our experiments γ -MSH precursor did not significantly induce steroidogenesis in the absence of ACTH it is uncertain whether the γ -MSH precursor has a direct steroidogenic action.

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Spleen colony-forming cell as common precursor for tissue mast cells and granulocytes

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The haematopoietic stem cells which produce colonies in the spleen of irradiated mice (CFU-S)¹ can differentiate into erythrocytes, granulocytes, megakaryocytes and B lymphocytes^{2–4}. Although mast cell precursors are known to be present in the bone marrow⁵, spleen⁶, fetal liver⁷ and peripheral blood⁸ of mice, the relationship between the mast cell precursor and CFU-S has remained unclear. We have now made use of mice of two mutant genotypes to determine whether or not the tissue mast cell is a progeny of CFU-S. Giant granules of beige (C57BL/6-*bg^j/bg^j*, Chediak-Higashi syndrome) mice can be used for identification of the origin of both tissue mast cells⁹ and granulocytes⁹, and WBB6F₁-W/W^o mice are useful recipients because they lack tissue mast cells owing to a defect in mast cell precursors¹⁰. We injected the cells from a single spleen colony into each WBB6F₁-W/W^o mouse and demonstrated directly that the tissue mast cell is a progeny of CFU-S.

Mice of strain WBB6F₁ (WB-W/+X C57BL/6-W^o/+)_{F₁}-W/W^o and C57BL/6-*bg^j/bg^j*, +/+ were obtained from the Jackson Laboratory, Bar Harbor, Maine. As the haemoglobin pattern of WBB6F₁-W/W^o mice (diffuse, *Hbb^d/Hbb^d*) can easily be distinguished from that of C57BL/6 mice (sharp, *Hbb^s/Hbb^s*), it was used to identify the origin of erythrocytes¹¹. The experimental design is shown in Fig. 1. X-ray-irradiated (800 rad) C57BL/6-+/+ mice were injected with a mixture of bone marrow cells from beige and normal C57BL/6 mice. The irradiated C57BL/6 mice were killed 12 days after injection of bone marrow cells, spleen colonies were enucleated under the dissection microscope and cells from each colony suspended in serum from C57BL/6 mice. An aliquot of suspended cells was smeared, stained with Sudan Black B and counterstained with Giemsa solution to examine the proportion and type of granulocytes (beige or normal)⁹. The rest of the cells were injected into a WBB6F₁-W/W^o mouse intravenously. A blood sample was obtained from a retroorbital sinus 15 weeks after the cell injection. Haemoglobin pattern was determined by cellulose acetate electrophoresis after modification of haemoglobin with cystamine¹² and smears were stained with Sudan Black B to follow the appearance of beige-type granulocytes. After the examination of peripheral blood, the WBB6F₁-W/W^o recipient mice were killed and tissue distribution of mast cell types was determined (Table 1). The interval of 15 weeks between cell injection and killing of the recipients was chosen because previous experiments showed that the number of mast cells increased in tissues of WBB6F₁-W/W^o mice until the fifteenth week after the bone marrow transplantation^{6,10}.

Table 1 Type of mast cells in tissues of WBB6F₁-W/W^o mice 15 weeks after injection of a single spleen colony each

Tissues	No. of mice in which mast cells of each type appeared			
	Beige type only	Normal type only	Both types	Total
Skin	0	0	0	0
Mesentery	0	2	0	2
Caecum	2	4	0	6
Forestomach	2	6	2	10
Glandular stomach	3	7	2	12
Spleen	10	7	3	20
Whole body*	10	9	4	23

Tissues of 106 WBB6F₁-W/W^o mice were examined. Spleen, stomach, caecum and a piece of dorsal skin were removed and fixed in 10% buffered formalin (pH 7.2). The stretched preparation of the mesentery was also fixed in 10% formalin. Tissues were embedded in paraffin; sections (5 µm thick) and stretched mesentery were stained with acidified toluidine blue (pH 3.0). Criteria to determine the appearance of mast cells in the skin, mesentery, forestomach and glandular stomach are described elsewhere⁶. Mast cells were considered to have developed in the spleen when more than 20 mast cells were detected per longitudinal section.

* No. of mice in which mast cells appeared in more than one of above tissues.

A total of 117 discrete colonies were enucleated from the spleen possessing 1–3 colonies which developed after injecting the mixture of 10⁴ beige and 10⁴ normal bone marrow cells. In 36 out of 117 colonies, more than 10% of cells in one colony were granulocytes. Beige-type granulocytes with giant sudanophilic granules were predominant (>90%) in 19 out of 36 colonies, and normal-type granulocytes predominated in 16 colonies. A considerable number of granulocytes of both types were observed in only 1 of 36 colonies (15% beige type, 85% normal type). Thus, each spleen colony could easily be identified as from either a beige C57BL/6 mouse or a normal C57BL/6 mouse.

One hundred and six WBB6F₁-W/W^o mice which each received cells from a single spleen colony survived for 15 weeks. A change of haemoglobin pattern from diffuse to sharp was detected in 3 out of 106 WBB6F₁-W/W^o mice. Only 1 of 106 WBB6F₁-W/W^o mice showed beige-type granulocytes (3%) in the peripheral blood. As shown in Table 1, mast cells appeared in more than one of the tissues examined in 23 out of 106 WBB6F₁-W/W^o mice. All the mast cells scored were of either beige or normal type in 19 out of 23 mice. The type of granulocytes in the original spleen colony had been determined in 10 of 19 such cases. The relationship between the type of granulocytes and that of the mast cells is shown in Table 2. The type of the

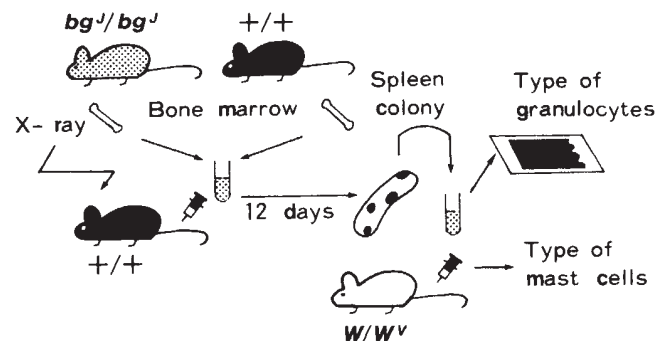


Fig. 1 Experimental design for comparison of the type of granulocytes in a spleen colony with that of tissue mast cells which appeared in WBB6F₁-W/W^o mouse after injection of cells from each colony.

Table 2 Comparison of the type of granulocytes in a spleen colony with that of mast cells which developed in tissues of a WBB6F₁-W/W^v mouse after injection of cells from each colony

Mouse no.	Type of granulocytes (beige:normal)	Type of mast cells (beige:normal)			
		Spleen	Glandular stomach	Forestomach	Caecum
2410	100:0	152:0	ND	ND	ND
2418	92:8	198:0	ND	ND	ND
2428*	6:94	0:317	0:253	0:48	0:383
3011	100:0	111:0	ND	ND	ND
3189	3:97	ND	0:354	ND	ND
3638	100:0	136:0	ND	ND	ND
4141*	100:0	373:9	64:0	120:0	38:0
4145	100:0	615:0	ND	ND	ND
4149	0:100	0:650	ND	ND	ND
4153	0:100	0:87	ND	0:381	ND

Mast cells did not develop in the mesentery and skin of all mice listed above. ND, mast cells did not develop.

* Haemoglobin pattern changed from diffuse to sharp in mice 2428 and 4141. Beige-type granulocytes (3%) appeared in the peripheral blood of mouse 4141.

Table 3 Development of mast cells in WBB6F₁-W/W^v mice 15 weeks after injection of various cells

Group no.	Origin of injected cells	No. of mice examined	No. of mice in which mast cells of each type appeared*			
			Beige type only	Normal type only	Both types	Total
1	Spleen fragment†‡ without colony	18	0	0	0	0
2	Colony from spleen‡§ with 1-3 colonies	106	10	9	4	23
3	Colony from spleen with 7-11 colonies	69	6	5	18 #	29**

* Mast cells appeared in more than one of tissues listed in Table 1.

† Areas of spleen other than colonies were excised from the spleen with 1-3 colonies.

‡ The mixture of bone marrow cells from beige (10⁴) and normal (10⁴) C57BL/6 mice was injected to make colonies.

§ Data from Table 1.

|| The mixture of bone marrow cells from beige (2×10⁴) and normal (2×10⁴) C57BL/6 mice were injected to make colonies.

P < 0.05 by χ^2 test, when the frequency of mice in which mast cells appeared was compared between groups 1 and 2.

* P < 0.01, when the frequency of mice in which both types of mast cells appeared was compared between groups 2 and 3.

** P < 0.01, when the frequency of mice in which mast cells appeared was compared between groups 2 and 3.

granulocytes and that of the tissue mast cells were exclusively consistent in all cases.

In the above experiment, mast cells appeared in only 23 of 106 WBB6F₁-W/W^v mice after injecting cells from an individual spleen colony. Two experiments were carried out to obtain more information on this low acceptance of spleen-colony cells. First, as there is a possibility that colonies were not excised precisely from the spleen, cells from the other areas of the spleen (over a diameter of about 2 mm) rather than the colony were injected to WBB6F₁-W/W^v mice. No mast cells appeared in the examined tissues of the W/W^v recipients 15 weeks after the cell injection (Table 3). Second, in the experiment shown in Tables 1 and 2, discrete colonies were enucleated from the spleen possessing 1-3 colonies to minimize contamination of cells derived from more than two colonies. However, the number of mast cell precursors per colony might be dependent on the number of colonies per spleen because there are controversial reports about the effect of graft size on proliferation and differentiation of CFU-S^{13,14}. Although the efficiency of mast cell development increased after injection of an individual colony enucleated from the spleen possessing 7-11 colonies, both beige and normal types of mast cells appeared in more than half cases (Table 3). The latter result illustrates the difficulty of enucleating an individual colony from a spleen with 7-11 colonies, so the increased efficiency of mast cell development could not simply be attributed to the effect of graft size.

The present results directly demonstrate that precursors of tissue mast cells are generated within a spleen colony produced by CFU-S. Because the type of granulocytes in the colony matched the type of mast cells in tissues of WBB6F₁-W/W^v mice, CFU-S seems to be a common precursor for granulocytes and tissue mast cells.

Although a considerable proportion of granulocytes was observed in only 36 out of 117 spleen colonies examined, most of the colonies contained a considerable proportion of erythroid cells. Since Wu *et al.*² have shown that erythroid cells and granulocytes in the individual spleen colony are derived from a single CFU-S, at least a portion of CFU-S seems to have the potential to differentiate into erythrocytes, granulocytes and tissue mast cells. In fact, after injection of a beige-type spleen colony into a WBB6F₁-W/W^v mouse (4141 in Table 2), haemoglobin pattern changed from diffuse to sharp, beige-type granulocytes appeared in the peripheral blood, and beige-type mast cells developed in the spleen, stomach and caecum.

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Immunochemical evidence for hybrid sialoglycoproteins of human erythrocytes

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The two major sialoglycoproteins of the human erythrocyte membrane (α and δ , glycophorins A and B^{1,2}) have identical amino acid sequences for the first 26 residues from the amino terminus, except that α expresses M or N blood group antigen activity whereas δ carries only blood group N activity. In addition, the asparagine at position 26 on α carries an oligosaccharide chain which is absent from the same position on δ . The two sialoglycoproteins differ in their remaining amino acid sequence and δ expresses blood group Ss activity. There are also variant sialoglycoproteins which have properties of both the α and δ molecules^{1,3} and may be hybrids of these. Using antibodies directed against different structural regions of the major sialoglycoprotein α , we confirm here that two variant erythrocytes (Miltenberger class V (MiV) and Ph) contain hybrid sialoglycoprotein molecules (Fig. 1). These hybrid sialoglycoproteins arise from cross-over events between the genes coding for α and δ . It is suggested that the two genes are closely associated in the order α, δ (5' $\rightarrow$ 3') on the chromosome.

The two antibodies used in this study were R18 and I16. R18 is specific for a portion of the extracellular region of α and I16 for the intracellular portion (and/or intramembranous portion; see Fig. 1). The R18 antibody (LICR.LON/R18) is a mouse monoclonal antibody that reacts with untreated and trypsin-treated normal erythrocytes but does not react with En(a-) erythrocytes which lack α (ref. 4). Trypsin removes part of the extracellular region of α , thus the binding site of the antibody lies between the extracellular residues 40 and 72 (ref. 4). I16 was produced in a rabbit by repeated subcutaneous injections of partially purified sialoglycoprotein α in Freund's complete adjuvant. The antigen was partially purified by butanol extraction of erythrocyte membranes⁵, followed by chromatography on Sepharose 6B equilibrated in 5 mM sodium phosphate pH 8.0, 0.1% Ammonyx LO⁶. The rabbit was bled 7–10 days after the fourth and subsequent injections. The antibody was shown to be specific for α by two-dimensional immunoelectrophoresis and immunoprecipitation using detergent-solu-

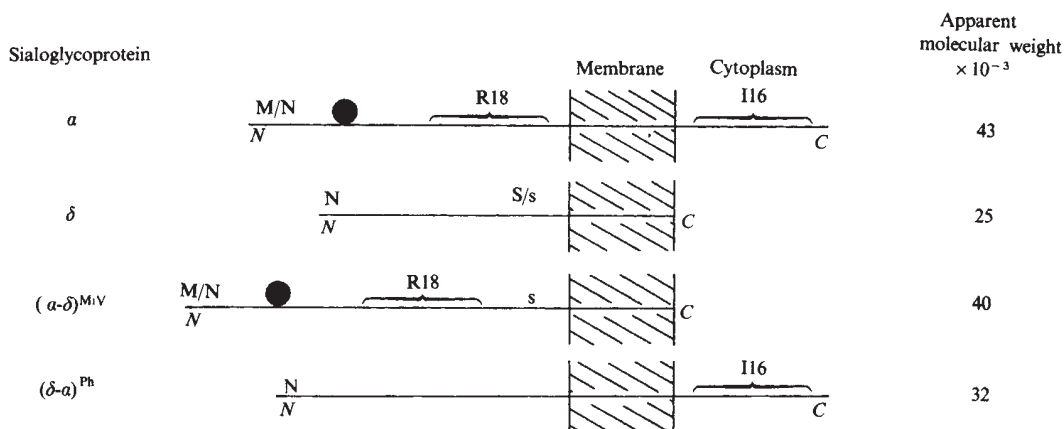
bilized radioiodinated erythrocytes. To remove antibodies specific for the extracellular portion of α , the antiserum was repeatedly absorbed with intact erythrocytes until no haemagglutination activity remained, and the amount of solubilized α immunoprecipitated remained unchanged. This antibody therefore recognizes determinants between residues 73 and 131 of α (ref. 7).

The two antibodies were used to immunoprecipitate membrane proteins of normal and variant human erythrocytes which had been labelled with ¹²⁵I using lactoperoxidase. The variant erythrocytes studied were MiV erythrocytes from both homozygous⁸ and heterozygous individuals¹, and erythrocytes heterozygous for the Ph condition³. These variants contain abnormal sialoglycoproteins designated (α - δ)^{MiV} in MiV erythrocytes¹ and (δ - α)^{Ph} in Ph erythrocytes³. Figure 2 (f, g, i, k) shows that the (α - δ)^{MiV} component is immunoprecipitated by R18 but not by I16. This sialoglycoprotein therefore contains an antigenic determinant of α in the extracellular region between residues 40 and 72, but lacks the determinant recognized by I16 in the remaining C-terminal portion. Previous evidence suggested that (α - δ)^{MiV} has features of both α and δ components^{1,8} as it carries weak blood group M, N antigen activity and has the characteristic trypsin sensitivity of normal α , but also carries blood group s activity which is associated with α ^{9,10}. The present results therefore provide independent confirmation that the variant sialoglycoprotein of MiV erythrocytes is a hybrid molecule consisting of the amino-terminal portion of α , and the carboxy-terminal portion of δ , or more accurately an unusual allele of δ ^{1,8}.

Figure 2 (h, l) shows that the (δ - α)^{Ph} component of Ph erythrocytes is immunoprecipitated by I16 but not by R18. The (δ - α)^{Ph} sialoglycoprotein therefore contains an antigenic determinant of α in the intracellular or intramembranous regions, but does not contain the extracellular determinant recognized by R18. We have previously suggested that the (δ - α)^{Ph} component contains the amino-terminal portion of δ as it is trypsin resistant, lacks an N-glycosidically linked oligosaccharide and blood group M activity, but carries blood group N activity³. The present data confirm that the (δ - α)^{Ph} component has an amino-terminal portion which differs from that of normal α , and conclusively shows that the component contains a determinant of the carboxy-terminal region of α . This provides strong evidence that the (δ - α)^{Ph} component is a hybrid molecule consisting of the amino-terminal portion of δ and the carboxy-terminal portion of α .

We suggest that the hybrid molecules arise by a mechanism similar to that which produces the abnormal Lepore-type haemoglobins^{11,12}, which occur when misalignment of genes leads to unequal crossing-over and generates new genes with characteristics of both the parent genes. The misalignment of genes coding for α and δ may result from the sequence

Fig. 1 Schematic diagram of the structures of normal and variant sialoglycoproteins. N and C represent the amino and carboxy termini of each sialoglycoprotein; M, N, S, s represent the blood group antigens associated with each molecule. The filled circle shows the approximate position of the asparagine-linked oligosaccharide when present. This oligosaccharide has been shown to be present on (α - δ)^{MiV} using a lectin binding technique³ with the *Phaseolus vulgaris* lectin (phytohaemagglutinin, data not shown). Also shown are the approximate locations of the antigenic sites recognized by the antibodies R18 and I16. The apparent molecular weights are derived from the electrophoretic mobility of the proteins on SDS-polyacrylamide gels run in the same conditions as those described in Fig. 2 legend.



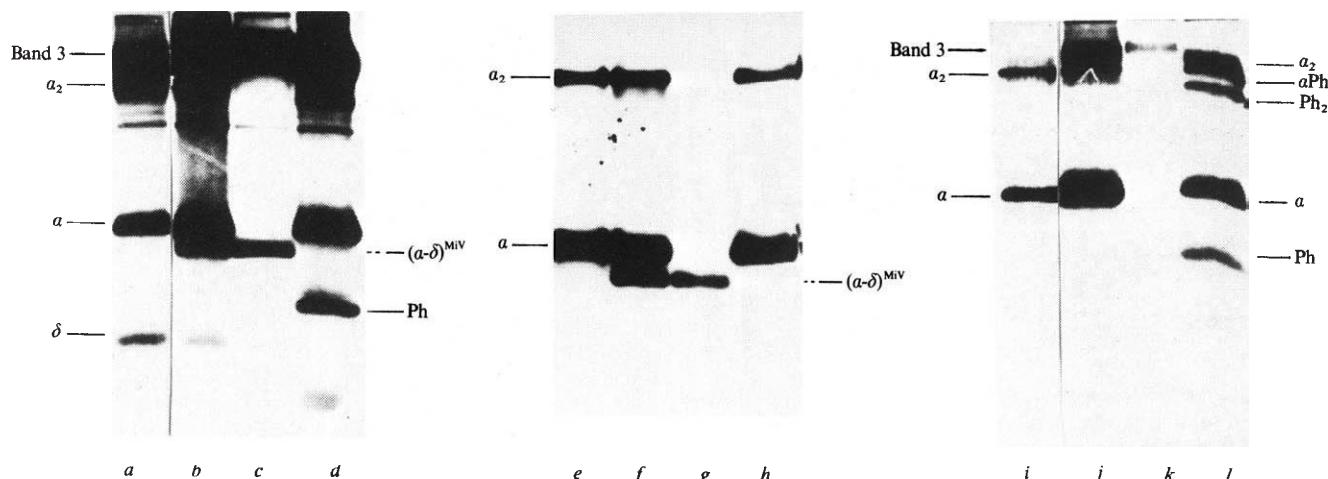


Fig. 2 Immunoprecipitation of labelled sialoglycoproteins from variant erythrocytes. Intact erythrocytes from donor M.P. (Ph variant), donor L.R. (heterozygous for the MiV phenotype), donor F.M. (homozygous for the MiV phenotype) and a normal donor were radioiodinated using lactoperoxidase¹. The cells were washed and solubilized in 4 vol of 150 mM NaCl, 50 mM Tris-HCl pH 8.0, 1% w/v Na deoxycholate¹⁶. When using R18 the solubilized sample was initially treated with mouse non-immune serum for 1 h, followed by rabbit anti-mouse IgG for 30 min and 200 μ l of a 10% suspension of fixed *Staphylococcus aureus* (strain Cowan I) for a further 30 min, all at 0 °C. The sample was then centrifuged and 10 μ l of R18 antibody was added to the supernatant, followed by 10 μ l of rabbit anti-mouse IgG (1 mg ml⁻¹) and 50 μ l of 50% Protein-A Sepharose (Pharmacia); times and temperature as for the initial treatment. The mixture was centrifuged and the pellet washed three times with 0.15 M NaCl, 5 mM EDTA, 0.5% Triton X-100, all at 0 °C. The pellet was then suspended in 20 mM Tris pH 8.0, 5 mM EDTA, 5% SDS, 10% glycerol, 0.1 mg ml⁻¹ bromophenol blue, 2% β -mercaptoethanol, boiled for 3 min, centrifuged and the supernatant electrophoresed in a 10% slab gel with a 5% overlay, using the buffer system of Laemmli¹⁷. When using I16 antibody the sample was first treated with normal rabbit IgG and *S. aureus* as above. The supernatant was immunoprecipitated with 10 μ l of I16 followed by 50 μ l protein-A Sepharose and the pellet treated as above. The SDS-polyacrylamide gels were run overnight and fixed in methanol/acetic acid/water (5:1:5) for 30 min, washed, dried and exposed to Kodak No-screen film for 1–5 days. *a–d*, Autoradiographs of radioiodinated intact erythrocytes before immunoprecipitation. *a*, Control erythrocytes; *b*, MiV heterozygous erythrocytes; *c*, MiV homozygous erythrocytes and *d*, Ph erythrocytes. *e–h*, Autoradiograph of ¹²⁵I-labelled components immunoprecipitated using R18. *e*, Control erythrocytes; *f*, MiV heterozygous erythrocytes; *g*, MiV homozygous erythrocytes and *h*, Ph erythrocytes. *i–l*, Autoradiograph of ¹²⁵I-labelled components immunoprecipitated using an IgG fraction of I16 antibody. *i*, Control erythrocytes; *j*, MiV heterozygous erythrocytes; *k*, MiV homozygous erythrocytes and *l*, Ph erythrocytes. The apparent molecular weights of the monomer bands are given in Fig. 1. α_2 , α Ph and Ph_2 represent the dimer of α , the complex of α with Ph and the dimer of Ph respectively. The band in lane *k* represents nonspecific background precipitation of band 3.

homology of the first 26 residues of the two proteins^{9,10,13}. It follows from this interpretation of the origin of the hybrid sialoglycoproteins that the genes coding for α and δ are closely linked on the same chromosome. Furthermore, the gene coding for α must be on the 5' side of that coding for δ as the event which gives rise to the α - δ hybrid sialoglycoprotein ((α - δ)^{MiV}) also results in the absence of both normal α and δ ^{1,8}, a situation similar to that occurring in Lepore-type haemoglobins. Conversely, the event which gives rise to a δ - α hybrid would not exclude normal α and δ , a situation analogous to the anti-Lepore haemoglobins. The (δ - α)^{Ph} component, a δ - α hybrid sialoglycoprotein, is associated with the presence of α but not δ ³. This absence of δ is unexpected and we have attributed it to a cross-over event between a normal chromosome containing α and δ and one lacking δ . The latter are common in the black African population from which the variant originated¹⁴.

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Note added in proof: The genes giving rise to the MN and Ss antigens and thus α and δ have recently been assigned to the q28 or q31 region of chromosome 4 (ref. 15).

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Properties of postsynaptic channels induced by acetylcholine in different frog muscle fibres

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Skeletal muscles in the frog are composed of two distinct classes of muscle fibre: fast muscle fibres capable of propagating action potentials and twitches, and slow muscle fibres normally unable to generate action potentials or twitches^{1,2}. In addition, amphibian muscles contain a spectrum of 'intermediate' fibres whose structural and functional properties lie between those of fast and slow fibres^{3–6}. Much is now known about the characteristics of the channels opened by the transmitter acetylcholine (ACh) acting on the membrane of fast fibres^{7,8}, but the molecular action of ACh on the other fibre types is only poorly understood. We report here the existence of a muscle in the mandibular arch of the frog in which most, if not all, the fibres are multiply innervated and are capable of eliciting action potentials. We also report that the channels induced by the transmitter on the synaptic membrane of fast, slow and submaxillary muscle fibres differ in their lifetimes and conductances.

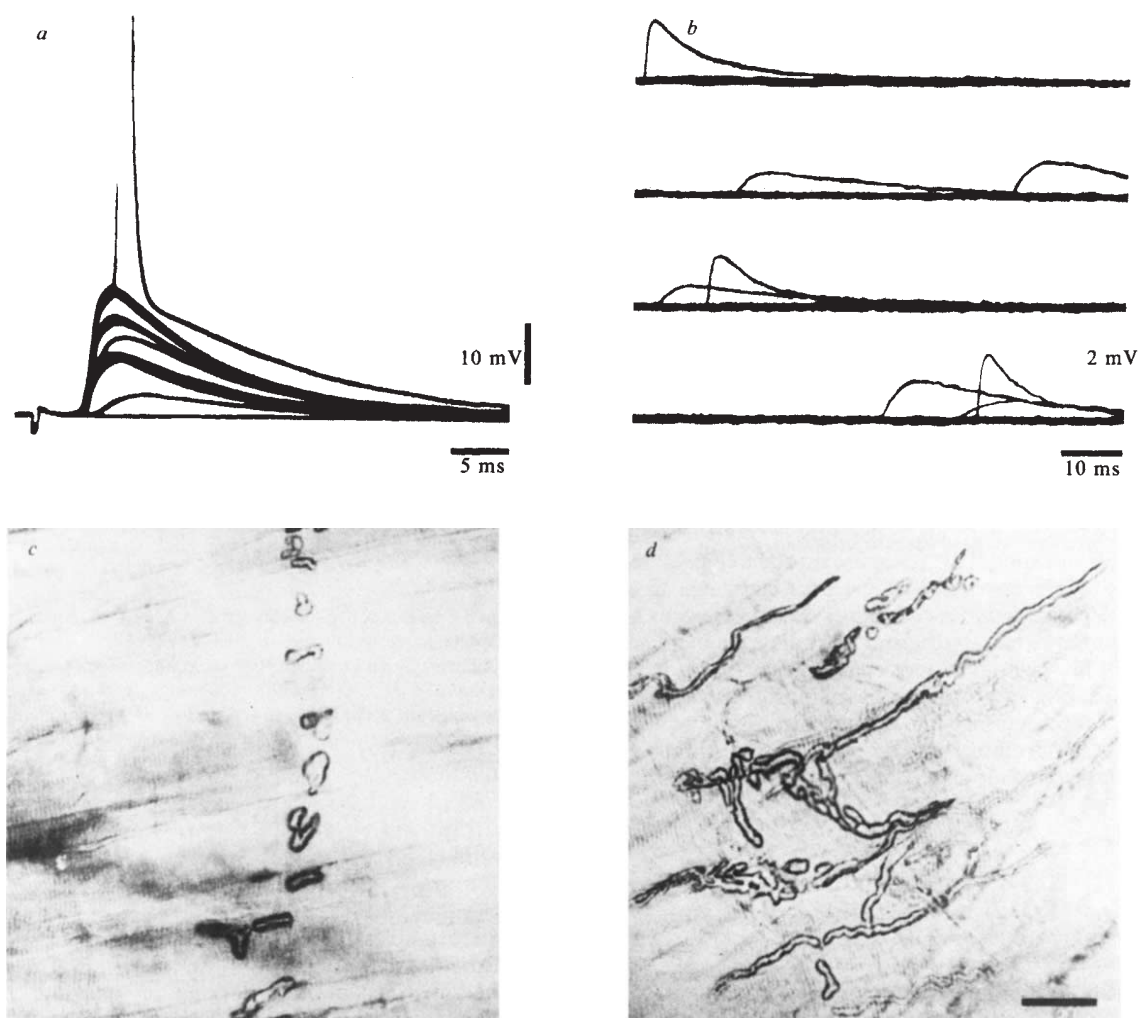


Fig. 1 Endplate potentials recorded intracellularly from a fibre in a submaxillary muscle in Ringer with 5.4 mM Ca^{2+} . The muscle was preincubated in 2 M formamide for 90 min to block the contractile responses²³. *a*, The superimposed records show five components obtained by increasing progressively the strength of the stimulus applied to the submaxillary nerve. An action potential was elicited by the compound endplate potential (Resting potential, 85 mV, temperature, 22 °C.) *b*, Miniature endplate potentials recorded from a fibre in the same muscle as in *a*. *c*, *d*, Partial views of two different regions of the same submaxillary muscle where cholinesterase was stained with Karnovsky and Roots method²⁴. Scale bar, 20 μm .

Experiments were performed on fast and slow muscle fibres of the pyramidal muscle⁹, and on the fibres of the submaxillary muscle⁹ of the frog *Rana temporaria*. The muscles, with their nerve supply, were isolated and bathed in normal Ringer solution (in mM: NaCl 115, KCl 2.5, CaCl_2 1.8; buffered with HEPES at pH 7.3), or in low Ca^{2+} -high Mg^{2+} Ringer (same as normal, but CaCl_2 0.3 mM and MgCl_2 5 mM), kept at about 6 °C. The different types of muscle fibres were identified by one or more of the following characteristics: passive and active electrical membrane properties, innervation pattern and contractile behaviour^{1-6,10,11}.

While surveying the ACh sensitivity and distribution of cholinesterase in frog muscle, Couteaux and Miledi (unpublished) found that in the submaxillary muscle the fibres were innervated at multiple sites and were capable of propagating action potentials. We decided to examine the muscle in more detail and, while the work was in progress, Kordylewski^{5,6} reported a morphological study of this muscle, which he calls sub-mandibular.

Muscle fibres in the submaxillary muscle are 8–16 mm long and extend from a narrow central tendon to the jaw. They are relatively small (5–50 μm diameter) and even after impalement with two low-resistance microelectrodes their input resistance is

several M Ω . All the fibres examined are able to generate an action potential when stimulated, directly with an intracellular microelectrode or indirectly through its motor nerve (Fig. 1*a*), and the action potential triggers a phasic contraction. All the muscle fibres are innervated by the submaxillary nerve, but the posterior part of the muscle is also innervated by the subhyoidus nerve. When the submaxillary nerve is stimulated, alone or together with the subhyoidus nerve, with stimuli of increasing intensity, the endplate potentials generated are made up of several discrete components (Fig. 1*a*), indicating that individual muscle fibres are innervated by several axons. Miniature endplate potentials are of low frequency and can be recorded anywhere along the muscle fibres down to their tendon ends. Their amplitude and time course are very variable (Fig. 1*b*), as would correspond to local events originating at various distances from the recording microelectrode.

As expected from the electrophysiological experiments, histochemical staining revealed synaptic contacts all over the muscle. Endplates vary in shape from simple small bouton-like contacts to long branched ones (Fig. 1*c,d*). Individual muscle fibres have 5–10 endplates distributed along their length, and both bouton and branched types seem to coexist on the same fibre.

Table 1 Characteristics of the synaptic ACh-activated channels obtained from power spectra of endplate current fluctuations

Muscle fibre type	τ noise (ms)	γ (pS)
	Mean $\pm$ s.e. (range)	Mean $\pm$ s.e. (range)
Fast	4.78 $\pm$ 0.28 (3.8–5.89) n = 7	16.09 $\pm$ 0.58 (14.08–18.12) n = 6
Submaxillaris	6.87 $\pm$ 0.4 (5.9–8.85) n = 9	13.22 $\pm$ 0.73 (9.82–15.42) n = 7
Slow	14.8 $\pm$ 0.64 (12.3–17.68) n = 8	7.76 $\pm$ 0.57 (5.81–9.75) n = 7

n , Number of fibres; clamp potential, -90 mV; temperature 4 – 6 °C. τ , mean lifetime of channel; γ , mean conductance of single channel.

The time course of endplate currents induced by nerve stimulation was studied under membrane voltage clamp. Because both slow and submaxillaris muscle fibres have distributed innervation, it is necessary to discriminate synaptic events arising at the site of recording from those occurring elsewhere. One way of achieving this is to place the muscle in low Ca^{2+} –high Mg^{2+} solution and apply Ca^{2+} ionophoretically to a small region between the two microelectrodes used for clamping. In these conditions, nerve impulses continue to invade the nerve terminals but fail to release transmitter, unless an endplate lies close to the Ca^{2+} pipette, in which case release occurs but only from this junction (see ref. 12). To avoid further complications arising from time dispersal in the release of transmitter quanta¹³, the efflux of Ca^{2+} from the pipette was adjusted so that nerve impulses released mainly single quanta. These unitary synaptic

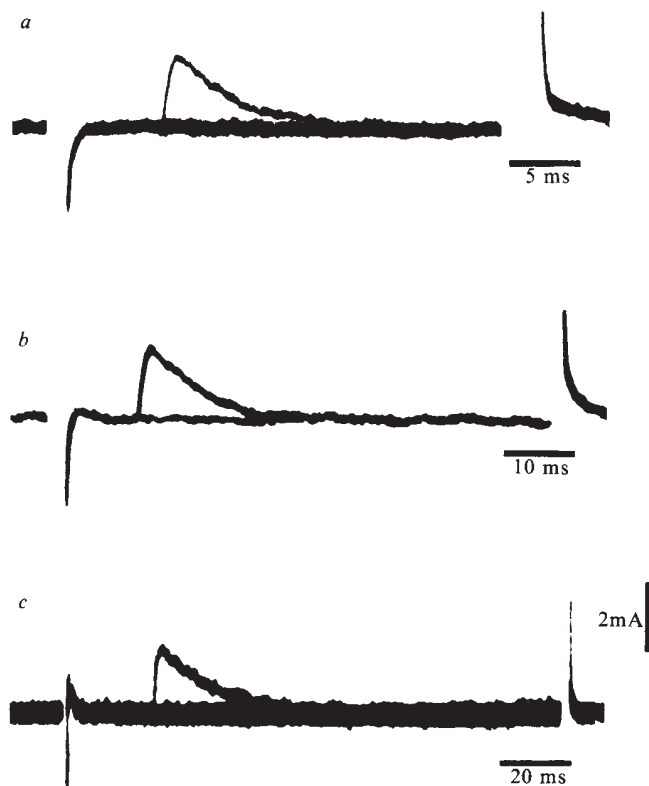


Fig. 2 Unitary (single quanta) endplate current responses to nerve stimulation, recorded from a fast (*a*), a submaxillaris (*b*) and a slow (*c*) muscle fibre. Muscles were in low Ca^{2+} –high Mg^{2+} solution at 6 °C. The artefact at the end of each trace indicates the start of the 800-ms pulse of Ca^{2+} used to restrict transmitter release to the endplate under study. Endplate currents were recorded under voltage clamp (-90 mV) through a 500-Hz low pass filter. The nerve was stimulated once every 1 or 2 s.

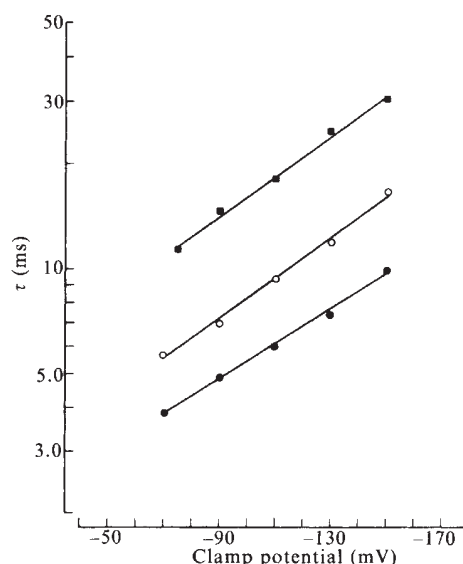


Fig. 3 Dependence of the time constant of decay of unitary endplate currents on membrane potential in a fast ($\bullet$), a submaxillaris ($\circ$) and a slow ($\blacksquare$) muscle fibre. Each point represents the mean of at least 10 unitary currents. An e -fold change in the time constant of decay was obtained when the muscle fibres were hyperpolarized by 84.5, 74.5 and 75.5 mV in the case of the fast, submaxillaris and slow muscle fibres, respectively. Normal Ringer at 6 °C.

events, examples of which are shown in Fig. 2, were then used to measure the time course of transmitter action.

The time constant of decay of unitary endplate currents, at a holding potential of -90 mV and at about 6 °C, was 5.0 ± 0.35 ms (mean $\pm$ s.e. of five fibres) in fast fibres and 14.67 ± 1.09 ms (six fibres) in slow fibres. Thus, the decay of transmitter action at slow muscle fibres is approximately three times slower than at fast fibres, a difference which resembles that found between fast and slow muscle fibres in the snake¹⁴ and in the iliofibularis muscles¹⁵. In submaxillaris muscle fibres the decay constant of unitary endplate currents varied over a wide range (from 6.5 to 13.17 ms, mean 8.87 ± 0.56 ms, 13 fibres), even among fibres within the same muscle. The decay phase of the unitary endplate currents of the fast, submaxillaris and slow muscle fibres was prolonged as the membrane was hyperpolarized¹⁶. Moreover, as indicated by the slope of the voltage-decay time constant relation, the voltage sensitivity was approximately the same in the three types of fibres (Fig. 3). In contrast, the decay of spontaneous miniature endplate currents in slow fibres of the snake seems to be much less sensitive to voltage than in fast fibres¹⁴. We do not know if this difference is entirely due to the different species used (frog as compared with snake) or whether other factors are also involved.

To see if the different time courses of unitary endplate currents were due to differences in the lifetime of the channels opened by ACh in the various types of muscle fibres, we analysed the current fluctuations produced by ionophoretic application of ACh to the neuromuscular junction^{7,8}. The mean lifetime (τ) of the junctional channels induced by ACh in submaxillaris muscle fibres was longer than in fast fibres, and the channel lifetime was longer still in slow fibres (Table 1). A close agreement was found between channel lifetime and the time constant of decay of unitary endplate currents in the three types of fibres, indicating that most of the ACh molecules, released from the motor nerve terminal at all these junctions, diffuse away from the ACh receptors or are destroyed by cholinesterase before they can exert repeated actions¹⁷.

The equilibrium potential for ACh action was found to be near zero in the different types of fibres, and the single channel conductance (γ) was estimated from both the variance of the current noise and the zero frequency asymptote of the power spectra^{7,8}. As shown in Table 1, junctional channels induced by ACh in fast fibres had larger conductance than the channels in submaxillaris fibres and these in turn had larger conductance than the channels in slow fibres. Here again, there seems to be a difference between frog and snake muscles, because fast and slow fibres in the snake appear to have the same single channel conductance¹⁴.

Knowing the single channel conductance and the amplitude of the unitary endplate current, an estimate can be made of the number of channels opened by the release of one transmitter package⁷. The amplitudes of unitary endplate currents in fast, submaxillaris and slow fibres were, respectively (in nA), $1.98 \pm$

0.35 ($n = 6$), 1.76 ± 0.12 ($n = 13$) and 1.36 ± 0.16 ($n = 6$). This suggests that the mean number of channels opened by a single transmitter packet is $\sim 1,370$ in fast fibres, 1,480 in submaxillaris and 1,950 in the slow fibres. More experiments are needed to substantiate these preliminary estimates further, and to determine the cause of such a difference.

We conclude that the membrane channels activated by ACh at the three types of muscle fibres studied differ in the time they remain open and also in their conductance. Some properties of frog muscle fibres are known to be under neural control¹⁸⁻²², and it is possible that the characteristics of the membrane channels induced by ACh on a given endplate are determined by the type of nerve which innervates it.

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Relative proteolysis of the fibrinogen B β chain by thrombin and plasmin as a determinant of thrombosis

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Thrombosis results, in part, from localized accumulation of fibrin, implying an imbalance between its rate of formation and dissolution. Astrup¹ postulated that patency of the vascular system depended on a dynamic equilibrium between constantly active coagulation and fibrinolytic systems. Reviews of this hypothesis have concluded that neither thrombin nor plasmin proteolysis makes a major contribution to fibrinogen turnover in normal individuals² and that the hypothesis of a dynamic equilibrium between clotting and lysis remains unproven³. Thus, the current view is that plasmin simply serves the function of a fibrinolytic enzyme digesting fibrin in the vascular system. Here, I promote the alternative view that the relative rates of proteolysis of the B β chain of fibrinogen by thrombin and plasmin determine the occurrence of thrombosis. This view is supported by recent data and can be used to make some readily testable predictions.

New biochemical information and technology now permit specific and quantitative analysis of the balance between thrombin and plasmin proteolysis of fibrinogen. Such analysis depends on measurement of the products of proteolysis which will briefly be reviewed. Thrombin cleaves fibrinogen to release sequentially fibrinopeptide A (FPA) and fibrinopeptide B (FPB)^{4,5}, implying two stages in fibrinogen-fibrin conversion with sequential activation of two sets of polymerization sites⁶. Removal of FPA exposes the A-binding domains in the NH₂-terminal region of fibrinogen which is now termed fibrin I. The

A-domains of fibrin I bind to COOH-terminal domains termed 'a' in an adjacent molecule⁷⁻¹⁰. The removal of FPB produces fibrin II, in which a second set of binding sites is exposed—the B-domains which bind to the b-domains of an adjacent molecule. Laurent and Blombäck¹¹ observed that fibrin I polymers were less compact than those formed by fibrin II. Fibrin I was thought to form primarily end-to-end polymers and fibrin II both end-to-end and side-to-side polymers. Shen et al.¹² confirmed and extended these findings. They postulated that removal of FPA results in slow assembly of thin fibrin fibres and that rapid assembly to thick fibres depends on the removal of FPB. Hantigan and Hermans proposed that formation of protofibrils is a prerequisite for removal of FPB by thrombin, a step which is itself a prerequisite for rapid fibre formation and gelation¹³. In the early stages of plasmin proteolysis of fibrinogen, two-thirds of the COOH-terminal A α chain and B β 1-42 from the NH₂-terminal B β chain are released to leave fragment X (refs 14-18). Hence, when fibrin I is the substrate, if the arginine (B β 14)-glycine (B β 15) bond is cleaved by thrombin, FPB is released and fibrin II formed, whereas if the arginine (B β 42)-alanine (B β 43) bond is cleaved by plasmin^{17,19,20} the products are B β 1-42 and fragment X (Fig. 1). Fragment X

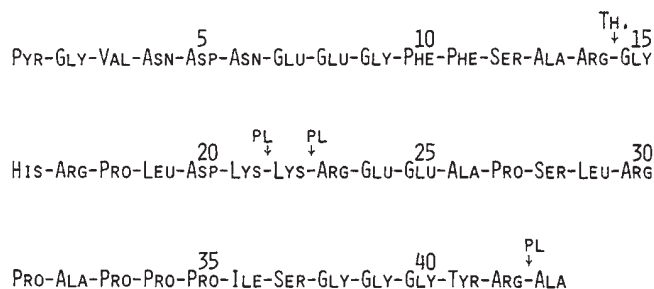


Fig. 1 Primary structure of the NH₂-terminal B β chain of fibrinogen. Bonds attacked by thrombin and plasmin are as indicated by Iwanaga et al.¹⁹ and Wallen²⁰.

formed from fibrin I will form a clot but this is only 1% as rigid as that formed from intact fibrinogen²¹, and α -polymer cross-links, a primary determinant of susceptibility to lysis by plasmin²², do not form with fragment X^{23,24}. Additionally, fibrin II is covalently cross-linked more rapidly than is fibrin I²⁵.

Some of these reactions can now be specifically quantified *in vivo*, as radioimmunoassays applicable *in vivo* have been developed for FPA reflecting fibrin I formation²⁶, thrombin-increasable FPB immunoreactivity (TIFPB) reflecting B β 1-42 and hence fragment X formation²⁷, and desarginine FPB reflecting fibrin II formation²⁸. The mean normal plasma FPA level is 0.6 pmol ml⁻¹ and elevated levels occur in acute venous and arterial thrombosis, pulmonary embolism, disseminated intravascular coagulation and other diseases²⁹⁻³⁶. It is evident that elevated FPA levels and hence fibrin I formation are not specific for thrombosis and that the effects of plasmin should also be considered. Peptide B β 1-42, reflecting the action of plasmin, can be identified in plasma as TIFPB²⁷. In normal individuals the concentration of TIFPB is always higher than that of FPA, with a ratio of ~1.5-3:1. A reproducible sequence of changes in the concentration of FPA and TIFPB occurred in the blood of patients who received an intra-amniotic infusion of hypertonic saline to induce abortion²⁷. First the FPA concentration increased and later the concentration of TIFPB rose, implying a sequence of fibrinogen proteolysis as shown in Fig. 2. If a single fibrinogen molecule is considered, in reaction (1), thrombin cleaves fibrinogen to yield free FPA and fibrin I monomer which polymerizes in reaction (2). Thrombin and plasmin then compete for the NH₂-terminal end of the B β chain (Fig. 1). If thrombin cleaves the B β chain before plasmin the products are FPB and fibrin II. If plasmin cleavage precedes that of thrombin the products are B β 1-42 and fragment X. Hence, plasmin cleavage of fibrin I serves as a major mechanism regulating the formation of fibrin II.

The facts that fibrin II forms more compact fibres than fibrin I and fibres with much greater tensile strength than fragment X and that elevated FPA levels are frequently found in conditions not associated with thrombosis prompt the hypothesis that the amount of fibrin II formed is important in thrombosis. The balance between the rates of fibrin II (reaction (3)) and fragment X (reaction (4)) formation would then determine the pathophysiological consequences of fibrin I generation. Predominant fibrin II formation results in thrombosis whereas predominant fragment X formation does not. The hypothesis explains and predicts phenomena, thus providing a means of testing its validity.

Phenomena predicted are that the ratio between peripheral blood FPA and TIFPB levels will demonstrate a greater association with thrombosis than FPA levels alone. Indices of fibrin II formation such as desarginine FPB levels may also be more closely associated with thrombosis than are indices of fibrin I formation. The thrombus itself should contain fibrin II. Although thrombosis does occur in the clinical syndrome of disseminated intravascular coagulation, there is often a marked dissociation between the degree of proteolysis of circulating fibrinogen and the extent of thrombosis found pathologically³⁷. According to the above hypothesis, this discrepancy would result from a successful body defence mechanism for coping with a large amount of fibrin I formed—transformation to the less harmful fragment X. Hence, one would predict that much fragment X would be formed in disseminated intravascular coagulation and there are reports of the presence of this molecule in the plasma of patients with this disorder³⁸.

The scheme shown in Fig. 2 implies that fragment X occurs as a polymer but the molecule seems to occur in monomeric form in plasma. Monomeric fragment X may be formed from depolymerization resulting from proteolysis or from plasmin proteolysis of fibrin I monomer. Ancrod and reptilase, which produce fibrin I^{39,40} and not fibrin II, do not cause thrombosis and in fact are used in its therapy. Additional mechanisms for the lack of thrombosis with these enzymes are that Ancrod degrades the COOH-terminal portion of the A α chain of fibrinogen and does

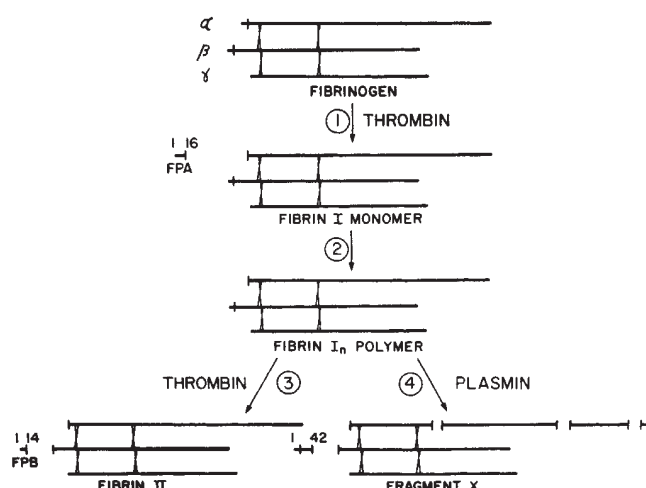


Fig. 2 Postulated scheme of fibrinogen proteolysis *in vivo*. Half fibrinogen molecules are depicted. Reaction (1), thrombin cleaves the A α chain of fibrinogen to produce free FPA and fibrin I monomer. Reaction (2), polymerization occurs. Reaction (3), thrombin cleaves fibrin I to produce free FPB and fibrin II. Reaction (4), plasmin cleaves fibrin I to produce B β 1-42 and fragment X. Evidence that reaction (2) precedes reaction (3) is from Blombäck *et al.*⁶. Evidence that reaction (4) occurs preferentially to plasmin proteolysis of fibrinogen is from H.L.N. *et al.*²⁷. Data have not been published on whether reaction (2) facilitates reaction (4).

not activate factor XIII and that neither enzyme aggregates platelets. Selectively increased thrombin concentration would favour reaction (3) and promote thrombosis, and congenital deficiency of antithrombin III, a condition expected to result in selective increase in thrombin concentration, is recognized as being associated with thrombosis^{41,42}. Selectively decreased plasmin formation would decrease the rate of reaction (4), and conditions with decreased plasmin formation have also been reported to be associated with thrombosis⁴³⁻⁴⁶. Selectively increased plasmin activity due to decreased activity of α_2 -antiplasmin may be associated with a bleeding tendency^{47,48}. Finally, it is evident that although fibrin is an important component of thrombi, both platelets and the vessel wall constituents have important roles in thrombosis. Hence, the proposed hypothesis concerning fibrin formation and thrombosis deals with only a part of the process.

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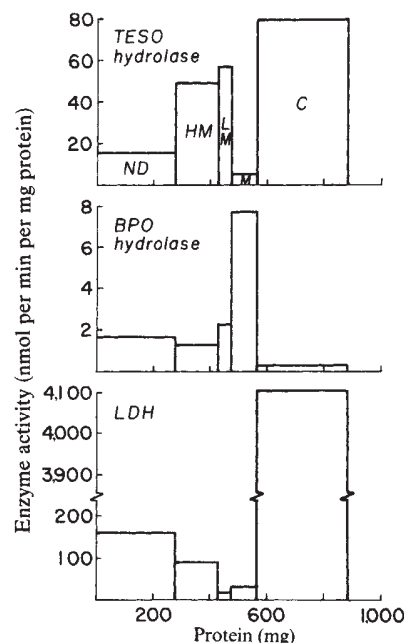


Fig. 1 Histograms of the subcellular distribution of epoxide hydrolase activity using TESO, *trans*- β -ethylstyrene oxide (upper), or BPO, benzo(a)pyrene 4,5-oxide (middle), as substrates and LDH, lactate dehydrogenase, with pyruvate as substrate (lower). Livers from 10-week old, male Swiss-Webster mice were rinsed in ice-cold 0.25 M sucrose and homogenized in 0.25 M sucrose/10 mM Tris-HCl, pH 7.4 buffer in a loose-fitting Potter-Elvehjem homogenizer to give a 10% w/v homogenate. The homogenate was centrifuged at 600g for 10 min, to give the nuclei and debris fraction (ND). The heavy mitochondria (HM) were pelleted at 6,500g for 10 min, the light mitochondria (LM) at 12,000g for 10 min, and the microsomes (M) at 100,000g for 60 min, leaving the cytosolic fraction (C) as the supernatant. Pelleted fractions were washed in the same conditions and resuspended in buffer (sodium phosphate, I = 0.2 M, pH 7.4 or Tris-HCl, 0.1 M, pH 9.0) for analysis. The ND, HM, LM, M and C fractions had 270, 160, 41, 87 and 320 mg of protein, respectively, from a preparation using three mouse livers. Separation of lysosomes and peroxisomes from mitochondria was achieved from livers, treated with Triton WR-1339 at 850 mg per kg 3.5 days before they were killed, by differential centrifugation as above followed by sucrose density gradient centrifugation¹⁵. The lysosomal and peroxisomal fractions had less than 2 and 28%, respectively, of the epoxide hydrolase activity in the crude mitochondrial fraction. The relatively high epoxide hydrolase activity in the peroxisomal fraction is probably due to mitochondrial contamination as the separation of these two fractions was not clear cut as determined by enzyme markers. Mitochondria were separated into inner membrane, outer membrane and the intermembrane and matrix fractions by swelling, shrinking and sonication, followed by sucrose density gradient centrifugation¹⁶. The values reported here are averages of two or three determinations from a preparation using three mouse livers. Of the total epoxide hydrolase activity present in the crude cell homogenate, 70 and 78% of the TESO and BPO hydrolysing activity is recovered in the various subcellular fractions.

Epoxide hydrolase activity in the mitochondrial fraction of mouse liver

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The intense interest in the metabolic fate of epoxidized xenobiotics is due to several factors. For instance, epoxides are often intermediates in the lipophile to hydrophile conversions necessary for the excretion of olefinic and aromatic compounds by living systems¹, and are widely encountered in man's diet from both natural and man-made sources. Some of these epoxidized compounds may alkylate proteins and nucleic acids and thus include some of the most potent cytotoxins, mutagens and carcinogens known². In mammals, epoxides may rearrange, deoxygenate to olefins, react with glutathione to form conjugates, or be hydrolysed by water to yield 1,2-diols with or without enzymatic catalysis^{1,3,4}. The enzymes which catalyse the formation of diols are known as epoxide hydrolases (EC 3.3.2.3), and their subcellular distribution is the subject of this report. Early data showed that styrene oxide hydrolase activity was associated with the microsomal subcellular fraction⁵. Epoxide hydrolase activity was subsequently demonstrated on the nuclear⁶, Golgi apparatus and plasma membranes⁷, and in the cytosol of the cell^{8,9}, leaving the mitochondria as the last major cellular organelle assumed to be devoid of epoxide hydrolase activity. We now report strong evidence for the occurrence of substantial epoxide hydrolase activity in the mitochondria.

While studying the subcellular distribution of the microsomal epoxide hydrolase, Oesch *et al.*⁵ reported substantial styrene oxide hydrolase activity in a crude mitochondrial fraction prepared in isotonic KCl. However, the same study showed that mitochondria prepared in isotonic sucrose had low styrene oxide

hydrolase activity which was not significantly different from that of the microsomal marker enzyme glucose-6-phosphatase. Analogous subcellular distribution studies in this laboratory using an epoxidized terpene or fatty acid as substrate showed high epoxide hydrolase activity in the mitochondrial fraction^{10,11}. However, in the case of these two substrates, the specific activity of the hydrolase was increased rather than decreased by repeated washing of the mitochondria. As no marker enzymes were used in these studies it was not possible to show definitively the presence of epoxide hydrolase activity in the mitochondria. Thus, the following experiments designed to yield 'pure mitochondria' were performed.

Using the procedure described in Fig. 1 legend, the purity of the mitochondrial and submitochondrial fractions was monitored with several enzymes. Subcellular distribution of epoxide hydrolase activity was monitored with benzo(a)pyrene 4,5-oxide (BPO)¹² and *trans*- β -ethylstyrene oxide (TESO)¹³ as

substrates. Significant TESO hydrolase activity, ~25% of the total recovered in the subcellular fractions, was observed in the mitochondrial fraction (Fig. 1), although the highest level was found in the cytosol. The subcellular distribution of epoxide hydrolase activity is very similar when *trans*-stilbene oxide (TSO) is used as substrate. Both TESO and TSO are negligibly hydrated by the microsomal epoxide hydrolase and the immunologically similar nuclear epoxide hydrolase. In contrast, the mitochondrial fraction poorly hydrates BPO, a substrate widely used to monitor epoxide hydrolase activity. Further, the low specific activity of BPO hydrolase in the mitochondrial fraction indicates minimal contamination of this fraction by nuclear and/or microsomal membranes as BPO hydrolase is a good marker enzyme for the microsomal and nuclear membranes^{1,6}. Repeated washing of the mitochondrial fractions leads to a substantial decrease in BPO hydrolase specific activity and most of this activity is associated with the outer mitochondrial membrane. As styrene oxide and BPO hydrolase activity in the microsomes are immunologically identical¹⁴, our data support the hypothesis of Oesch¹ that styrene oxide hydrolase activity in the mitochondrial fraction is probably due to microsomes adhering to the outer mitochondrial membrane. In contrast, the TESO hydrolase specific activity in the mitochondrial fractions increases following repeated washings.

Using lactate dehydrogenase, a widely accepted marker for the cytosol, we demonstrated that epoxide hydrolase activity in the mitochondrial and nuclear fractions does not result from cytosolic contamination (Fig. 1). Results using additional marker enzymes including cytochrome oxidase¹⁵, NADPH cytochrome *c* reductase¹⁶, cytochrome P₄₅₀ (ref. 17), citrate synthase¹⁸ and adenylate kinase¹⁹ also support the relative purity of the subcellular fractions. Use of citrate synthase and adenylate kinase also showed that the cytosolic epoxide hydrolase activity does not arise from mitochondrial lysis during homogenization, as only 0.6 and 2% of the mitochondrial activity of these enzymes, respectively, was found in the cytosol.

Subsequent experiments using livers from mice treated with Triton WR-1339 (ref. 15) indicated that lysosomes and peroxisomes, monitored by acid phosphatase¹⁹ and urate oxidase²⁰, respectively, have minimal TESO hydrolase activity. As such, epoxide hydrolase activity in the mitochondrial fraction does not arise from contamination by these organelles. When mitochondria are disrupted, a preponderance of the epoxide hydrolase activity is associated with the matrix and intermembrane space fraction with TESO as substrate and less activity is associated with the inner and outer mitochondrial membranes. Most of the activity associated with either of these membranes can be removed with a single wash, indicating contamination by the matrix and intermembrane space fraction. The mitochondrial fraction is able to hydrate a wide variety of epoxidized compounds including epoxidized fatty acids and glycidal ethers, with a spectrum of activity similar to that of the cytosolic epoxide hydrolase, but distinct from that of the microsomal enzyme.

As the nuclei and mitochondria are excluded from the activation/deactivation systems used in many short-term mutagenicity assays²¹, these systems may not provide a valid picture of the ability of cells to degrade potentially mutagenic epoxides. However, this effect may be more quantitative than qualitative, because the mitochondrial epoxide hydrolase demonstrates a spectrum of activity similar to that of the cytosolic epoxide hydrolase.

The present study and published literature^{1,6,7,22} illustrate the apparently ubiquitous nature of epoxide hydrolases in cellular components. Although the information needed to understand fully the biological role of epoxide hydrolases is lacking, their ability to metabolize some xenobiotics is clear. The association of epoxide hydrolases with the mitochondria and nuclei of the cell may serve to protect the structural integrity of these organelles as well as their nucleic acid content. It was recently shown^{23,24} that mitochondrial DNA is more easily alkylated by polyaromatic hydrocarbons and their diol-epoxides than nuclear DNA. Such results are not altogether surprising because

of the nature of mitochondrial DNA and the oxidases occurring in the mitochondria^{25,26} which can activate the polyaromatic hydrocarbons to reactive electrophiles. Although the mitochondrial epoxide hydrolase activity reported here does not appreciably metabolize BPO, these epoxide hydrolases can potentially protect the mitochondria from those reactive epoxides which are substrates. Therefore, our study certainly illustrates the need for increased research on these important enzymes using a variety of potential substrates in all subcellular components.

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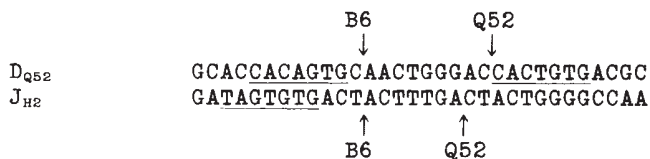
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Errata

In the letter 'Human immunoglobulin variable region genes—DNA sequences of two *V_κ* genes and a pseudogene' by D. L. Bentley and T. H. Rabbitts, *Nature* **288**, 730-733 (1980), Fig. 3 shows the DNA sequence of a human *V_κ* pseudogene (not *V_κ*).

In the article 'Sequence and organization of the human mitochondrial genome' by S. Anderson et al., *Nature* **290**, 457-465, line 56 on page 462 should read '... of the anticodon of mammalian mt RNAs is not known, it is ...'.

In the article 'Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes' by Y. Kurosawa et al., *Nature* **290**, 565-570, the arrows indicating recombination sequences in the text figure on page 570 were positioned incorrectly. The correct version is shown below:



Corrigendum

In the letter 'A nonrandom component in cosmic rays of energy $\geq 10^{14}$ eV' by C. L. Bhat et al., *Nature* **288**, 147-149 (1980), the RA values shown in Figs 2 and 3 have been found to be incorrect. The correct values are those obtained by subtracting the values given from 24 hours. This excludes the pulsar PSR 0525 + 21 as a possible origin. The results are consistent with a point-source origin in the RA range 20 ± 3 hours.

MATTERS ARISING

Fortuitous oxidations by methane-utilizing bacteria

A MAJOR thesis of Higgins *et al.*¹ is that the wide substrate specificity shown by methane monooxygenase (MMO) has developed because of its survival value to the species by enabling the organisms to use ancillary carbon or energy sources.

In support of their thesis, the authors state that "such extraordinary lack of substrate specificity would be extremely unusual if it were entirely fortuitous". There are, however, many examples of oxygenases and other enzymes which have a wide substrate range but which are only metabolically functional for a single substrate. For example, ribulose biphosphate carboxylase in all autotrophs has both carboxylation and oxygenation activities, the latter apparently leading to wasteful metabolism which has not been eliminated over a long period of biochemical evolution². Nitrogenase, an enzyme which reduces many triply bonded substrates, also produces dihydrogen as an intrinsic part of the reduction process, clearly a wasteful mechanism³. If there were some survival value to the species there would be a strong selective pressure on the organism to eliminate these 'side' reactions or to retain, evolve or acquire those enzymes that were capable of assimilating the product into biomass. Although it is possible that acetate could arise as a result of β -oxidation of 1-phenylheptane and ω -oxidation of higher alkanes and may therefore subsequently yield reducing equivalents, there is no direct evidence that any of the biotransformation products given in ref. 1, Table 1 are incorporated into biomass. Indeed, the biotransformation of most of these substrates results in a net loss of available energy to the cell because they require NADH for oxygenation to the product.

It is possible, for example, that the oxidation of toluene to benzoic acid may result in a net gain in reducing equivalents due to the activity of nonspecific alcohol dehydrogenases; however, no NADH is generated. As MMO requires NAD(P)H *in vitro*⁴⁻⁶ (and probably *in vivo* also), any oxidation that does not give rise to NAD(P)H may well be detrimental to the cell's overall energy balance. Furthermore, it has been calculated that the NAD(P)H-requiring MMO organisms are probably limited for NAD(P)H supply during growth on methane⁷. The fact that some of the products are certainly more toxic to the cell than the substrate also argues against the retention of a wide-specificity MMO. We therefore contend that these biotransformations, which are supposed to aid the survival of the

organisms, probably have exactly the opposite effect.

On closer examination of the paper quoted by the authors on their own work using whole cells, one observes that they were using suspensions containing 70–80 mg dry weight of organisms per 20 ml to effect the biotransformations of these compounds over a 15–24-h period. The high cell concentration necessary to detect oxidation is clearly unrepresentative of the situation in the biosphere.

Therefore, we feel that the arguments for the wide substrate specificity of MMO having "substantial metabolic significance" are invalid. We re-affirm our belief that all these oxidations are purely fortuitous⁸ and that there is no direct evidence for the oxidation of any substrate by MMO, other than methane, conferring a special advantage to the cell, whether it be as a supplementary carbon source or a provider of useful energy. We have always subscribed to the view that methanotrophs have a significant role in the oxidation of organic compounds and ammonia in the environment, but purely by a fortuitous mechanism and not one that has evolved or been retained to confer a substantial metabolic advantage to the cell.

Some methanotrophs, faced with a shortage of methane in the environment, may well oxidize any available ethane to acetate, but significant amounts of such acetate could be incorporated only by the type II methanotrophs (and not type I methanotrophs as the latter have an incomplete tricarboxylic acid cycle). Acetate could only serve as a source of carbon and not ATP because these organisms are unable to grow on acetate alone. Even so, it would still be necessary to generate NADH for ethane oxidation from the oxidation of methane to CO₂. But why is it necessary to do this for survival? All the methane oxidizers (where explored) seem to be able to form resting cells (cysts or exospores) which can survive in a hypobiotic state for years. Surely the elaboration of these protective mechanisms is far more relevant to survival than a catholic MMO enzyme able to scavenge trace amounts of carbon from the environment for the doubtful pleasure of making acetate?

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HIGGINS *ET AL.* REPLY—We do not accept the argument that there is nothing unusual about the extreme lack of specificity of some methane monooxygenases (MMO). Most oxygenases have a relatively narrow substrate range, some of them only acting on one or two very similar compounds. Even in the case of enzymes closely related to MMO, the microbial alkane hydroxylases, each one is restricted to quite a narrow band of chain lengths. The only oxygenase of which we are aware that approaches MMO in its 'catholic taste' for substrates is the mammalian liver P450 LM fraction¹. Here it is very probable that there has indeed been selection for broad specificity as this enzyme is essential to initiating processes for the removal of a wide range of toxic metabolites and xenobiotics from the body. In addition, in some methanotrophs the MMO seems to have a more restricted substrate range², showing that extreme lack of specificity is not a mechanistic dictum.

We accept that the lack of specificity of ribulose biphosphate carboxylase and nitrogenase leads to wasteful metabolism in some circumstances and various species have developed mechanisms to minimize this in the former case. Nevertheless, compared with MMO these are highly specific enzymes. Indeed, ribulose biphosphate carboxylase is specific for O₂ or CO₂.

In reply to the specific points raised by Stirling and Dalton:

(1) It is pointed out that many of the reactions catalysed are actually detrimental to the organisms because of reducing power consumption or the generation of toxic products. This point was made in our review. If this is the case, lack of development of more specific enzymes would seem to require some explanation. In fact, of course, this is something of a 'red herring' because most compounds of that type are not present in the environment in substantial amounts. Some have only recently been introduced as a result of the activities of the organic chemist. Therefore, we agree that many of these actual oxidations are probably purely fortuitous, although the ability to carry out some of these oxidations, we suggest, has been retained as a desirable characteristic. We did not imply that most of the reactions in our Table 1 lead to utilizable carbon or energy generation. The purpose of the table was simply to give an idea of the types of reactions catalysed. Such reactions may be of industrial value and may be involved in

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oxidation of a range of compounds in the environment.

The only substrates for MMO of significance *vis à vis* any selective value for broad-specificity enzymes are those which on subsequent metabolism yield acetate. There are two reasons for this. First, these compounds (long-chain alkanes, long-chain alkenes, phenylalkanes, carotenoids) are present in very substantial quantities in the environment; they are major components of biomass. Second, acetate once formed can act as a carbon source for methanotrophs and in the case of type II species which possess an intact tricarboxylic acid cycle, acetate can be oxidized to CO₂ yielding reducing power and ATP. It is well authenticated that acetate is extensively metabolized by type II species and leads to appreciable increases in growth yields when included in the medium³. Phenylalkanes are partially degraded by *Methylosinus trichosporium* to short-chain alkanolic acids and alkanes are oxidized to CO₂. The most likely mechanism for this is β -oxidation, the product of which is acetate. Further evidence for β -oxidation comes from the finding that C-odd phenylalkanes yield only C-odd phenylalkanoic acids and C-even phenylalkanes yield mainly C-even phenylalkanoic acids⁴. The example of 1-phenylheptane metabolism was given in our Table 1.

(2) There is accumulating evidence that methanotrophs possess other broad-specificity enzymes including secondary alcohol and aldehyde dehydrogenases^{5,6} that facilitate the further oxidation of products of MMO activity. Some of these enzymes, such as the secondary alcohol dehydrogenase discovered by the Exxon group, have no known function in C-1 metabolism.

(3) The environmental significance of reactions observed in our experiments using high cell densities is questioned. Of course, it is always difficult to extrapolate from laboratory experiments to the environment. It is not necessary to use such high cell densities to detect products, but clearly at very low cell densities product detection is difficult for technical reasons. We have used high cell densities to obtain in a short time sufficient material from small-scale incubations for both detection and chemical identification. In addition, because some of these substances are toxic at high concentrations, appropriate ratios of organisms to test compounds are necessary.

(4) We agree that the formation of exospores or cysts has great survival value. Would it not be advantageous, however, to be able to survive in a vegetative state using ancillary carbon and energy sources while your competitors are encysting?

(5) The fact that methanotrophs do not grow on acetate alone is not proof that acetate can act only as a carbon source. Acetate substantially increases yields of type II organisms on methane, suggesting

that NAD(P)H and/or ATP are generated from this compound. It seems likely that type II methanotrophs have retained an intact tricarboxylic acid cycle to facilitate acetate oxidation. The reasons for the inability to grow on acetate may well be found in the mechanisms of regulation of intermediary metabolism.

There are many examples of microbial co-oxidations in which ATP or NADH are generated from substances which are not growth substrates. Methanotrophs will not grow on formate but generate NADH (and possibly ATP) from this intermediate.

We suggest that more experimental work should be done to test our hypothesis that lack of specificity of MMO leads to useful supplementary metabolism and this is in progress in our laboratory.

Note added in proof: Dalton and colleagues have previously subscribed to the view that in the presence of methane small amounts of co-oxidizable compounds may well contribute significantly to the growth of methanotrophs and not just be merely oxidized without benefit to the organism^{7,8}.

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Cycloheximide inhibits DNA synthesis in SV40-transformed cells

IDE *ET AL.*¹ reported that DNA synthesis in an SV40-transformed human cell line (SV80) is not inhibited by the protein synthesis inhibitor cycloheximide. This is an apparent exception to the repeated observation that inhibitors of protein synthesis are effective inhibitors of DNA and RNA (mostly ribosomal) synthesis^{2,3}. Using various other cell types, both neoplastic and normal, we have found^{4,5} that the inhibitory effect of these drugs is also exerted on several reactions in both the salvage and *de novo* pathways of nucleotide biosynthesis, such as the formation of CTP from uridine and aspartate and the formation of GTP from hypoxanthine, adenosine, deoxyadenosine and formate.

CTP and GTP are not only required for RNA synthesis but are also intermediates in the formation of the deoxynucleoside triphosphate precursors, dCTP, dTTP and dGTP. Various lines of evidence emphasize the importance of maintaining normal pool sizes of these precursors for RNA and DNA synthesis^{6,7}, particularly for the latter, as the level of the deoxynucleoside triphosphates is very small in eukaryotic cells.

The SV40-transformed system could be pertinent to the question of whether DNA synthesis inhibition depends solely on the synthesis of some unknown protein(s) of short half life, in some way necessary for the polymerization reaction, or additionally on the levels of newly synthesized precursors. Thus, we had hoped to use the transformed cells to determine whether, in conditions in which DNA synthesis is not affected, the reactions leading to the biosynthesis of the precursors are similarly unaffected. Unfortunately, we are unable to confirm the observations of Ide *et al.* In our study, after addition of cycloheximide or puromycin in both the human SV80 line that Ide *et al.* used, and in an SV40-transformed mouse line (BALB/c SVA31), DNA synthesis was inhibited to the same extent as in the untransformed cell (BALB/c A31). In addition, all the effects on the nucleotide pool constituents that we observed with other cell types were seen with the SV40-transformed lines.

There is no apparent explanation for the differences between our results and those of Ide *et al.*, who suggest that the persistence in DNA synthesis in transformed cells is associated with the T-antigen content of SV40-transformed lines. T-antigen assays of our stocks showed that the BALB/c SVA31 and the SV80 lines are strongly positive whereas the untransformed BALB/c A31 is negative. We conclude that SV40-transformed cells are not exceptions to the statement⁸ that "inhibition of protein synthesis by any mechanism results in almost immediate interruption of DNA replication."

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Beaming of Jupiter's decametric radiation

POQUERUSSE AND LECACHEUX¹ reported the first direct measurement of the beaming of Jupiter's decametric radiation based on simultaneous observations of the jovian radio emission made with the Soviet spacecraft Mars 7, and at the ground level at Nancay, France on two different days. On one of them, 15 May 1974, activity was recorded at the spacecraft while none was observed at Nancay or at the University of Colorado site, and it was concluded that this effect was evidence of the narrow beaming of the jovian decametric radiation. We made complementary observations from the Maipú Radio Astronomy Observatory in Chile and from the University of Florida Radio Observatory: strong activity from Jupiter was recorded at the same time at a lower frequency and at a close frequency some 40 min earlier.

Table 1 shows the time and frequencies where the activity was recorded, including the spacecraft, Florida and Maipú. In Florida the emission was detected at 20.0 and 22.2 MHz, while in Maipú, Chile at 13.1, 16.7, 18.0, 22.2 and 27.6 MHz. Jovian activity was recorded at 22.2, 18.0 and 16.7 MHz and at the same time the spacecraft detected activity at 30 MHz while that at 27.6 MHz was recorded some 40 min earlier.

The antennas used were linearly polarized Yagis except for those operating at 13.1, 16.7 and 22.2 MHz in Chile, which were crossed polarimeter Yagis. The receiving, recording and calibrating system at both observatories were identical, each channel consisting of a modified commercial communication receiver tuned to the corresponding centre frequency with a fixed bandwidth of 4 kHz, a chart recorder with a time constant close to 1 s was used, although a supplementary recording was available for better resolution of individual bursts. Temperature-limited noise diodes were used as standard calibrator. Occasionally, no calibration was made during the observation—the galactic background noise serving as an auxiliary calibrator.

The flux density for each channel was determined following the standard procedure of the Florida–Chile group^{2,3}.

The flux densities of mean and peak pulses calculated for the observed activity at Maipú and Florida during 15 May 1974 are also included in Table 1. The emission recorded was mostly of the S-bursts type (millisecond pulses) and right-hand circularly polarized (16.7 and 22.2 MHz, Chile). The minimum detectable flux density (threshold) at Maipú and Florida was of the order of $2.5 \times 10^{-22} \text{ W m}^{-2} \text{ Hz}^{-1}$ at 22.2 MHz. The threshold of the University of Colorado spectrograph (7–41 MHz) was reported to be $5 \times 10^{-22} \text{ m}^{-2} \text{ Hz}^{-1}$ (ref. 1), that is well

Table 1 Summary of observations, 15 May 1974

Site	Frequency	Activity time		Flux density		Remarks
		UT		$(10^{-22} \text{ Wm}^{-2} \text{ Hz}^{-1})$		
Spacecraft	30	10.35	11.06	—	80	Ref. 1
Nancay	25–38	—	—	—	—	Ref. 1
Colorado	7–45	—	—	—	—	Observing time: 09.00–12.00
Florida	20.0	11.26	12.00	12.7	48.2	Jupiter transit: 13.03
	22.2	11.19	12.13	21.5	83.0	
Maipú	13.1	10.20	10.40	52.2	205.9	Jupiter transit: 12.11; sunrise: 11.26
	16.7	09.40	11.27	76.3	172.1	Right-hand polarized
	18.0	09.40	11.33	23.2	52.0	
	22.2	09.42	11.25	19.9	45.6	Right-hand polarized
	27.6	09.45	09.55	50.0	202.1	Antenna breakdown at 09.55

Observing frequencies, beginning and ending times of the activity, and mean and peak flux density measurements are given for each site where activity was recorded. At the highest frequency in Maipú (27.6 MHz), the antenna had a major breakdown at 09.55 so that the jovian activity was only recorded up to ~40 min before the spacecraft started recording the emission from Jupiter.

below the flux density of any mean pulse recorded in Chile or Florida (see table 1). The lack of correlation between the data obtained in Maipú and Florida suggests a strong ionospheric disturbance along the line of sight of Jupiter as seen from Florida. The same disturbance also seems to have affected the observations at Colorado, where Jupiter was being observed at a large zenith angle. It might even be possible to consider some scattering produced by field-aligned ionospheric irregularities.

We can thus add to Poquérousse and Lecacheux's result that Jupiter's strong beaming may hold only for a relatively narrow frequency bandwidth. As might be expected, the radiation beam appears

wider when all frequencies are considered rather than a single frequency.

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Corticosteroid-binding globulin and corticosterone interaction with progesterone receptors

IN their article¹ Al-Khouri and Greenstein claim to have shown “differences between progesterone receptors and CBG [corticosteroid-binding globulin] distribution in brain and uterus”. We would like to point out that, in their studies, they have failed to take into consideration binding of ³H-progesterone to other types of receptor sites, in particular glucocorticoid sites, and that for this reason they cannot conclude that “progesterone, the natural hormone, displaces the tritiated steroid from the receptor in brain and uterine cytosols more potently than does R5020”.

In the conditions used by Al-Khouri and Greenstein (non-ovariectomized animals, not primed with oestrogens), progesterone-binding sites (PR) are occupied by endogenous progesterone which cannot be exchanged with ³H-progesterone within 2 h at 0 °C without loss of

sites by degradation. Furthermore, even in the perfused brain and in optimal experimental conditions, the number of PR is low compared with the concentration of glucocorticoid binding sites (GR) and CBG. In the non-perfused uterus the relative quantity of PR compared with GR and CBG must be even smaller and ³H-progesterone binding corresponds to the total binding of PR, GR and CBG and not to PR uniquely. Thus, in the displacement studies, cold R5020 displaces ³H-progesterone primarily from PR and to a lesser extent from GR (note that competition of progesterone for GR is underestimated in screening assays because of trapping of progesterone by CBG) and cold corticosterone displaces ³H-progesterone from GR and CBG. This explains why corticosterone is more effective in the non-perfused uterus still rich in CBG.

The results would have been completely different with ³H-R5020 because, on account of its specificity, its absence of CBG binding and its much higher affinity for PR (slower dissociation of the receptor complex), progesterone can be nearly totally exchanged within 2 h at 0 °C.

^3H -R5020 thus measures virtually all PR and only PR^{2,3}.

Part of the aim of Al-Khouri and Greenstein's study was "to know the mechanism of action of progesterone in the brain". The progestin receptor measured with ^3H -R5020 has been shown to be implicated in progesterone activation of female sexual behaviour by comparing PR levels in conditions where progesterone is active or inactive in rats⁴ and guinea pigs^{5,6}.

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GREENSTEIN AND AL-KHOURI
REPLY—There is a striking difference between the brain and the uterus in terms of the specificity of ^3H -progesterone binding and we feel they should be discussed separately.

As regards the brain, we showed that progesterone was far more potent than was corticosterone in displacing ^3H -progesterone from its binding sites in the cytosol. As corticosterone has a very high affinity for its own receptor ($K_d \sim 10^{-9} \text{ mol l}^{-1}$)^{1,2}, one is constrained to conceive a situation in which corticosterone, but not progesterone, is denied access to the glucocorticoid receptor. Furthermore, progesterone has only one-third the affinity exhibited by corticosterone for the glucocorticoid receptor¹. Raynaud and Moguilewsky stated that ^3H -progesterone did not displace endogenous progesterone from the progestin receptor, although they did not specify the tissue. It has been shown elsewhere, using ovariectomized rats, that there is rapid exchange between ^3H -progesterone and the unlabelled hormone at the neural progesterone receptor³. Whether this is a progestin receptor is by no means clear. From our results⁴ the simplest conclusion is that ^3H -progesterone bound to a specific receptor from which it was displaced more readily by progesterone than by four other steroids.

In the uterus there is a completely different picture. We agree readily with Raynaud and Moguilewsky that there is extensive binding cross-reactivity between progesterone and corticosterone at their receptor sites. Indeed, this difference between brain and uterus is a central point of our paper. At this stage we

cannot distinguish between the many components in uterine cytosol to which ^3H -progesterone was bound. As there is ample evidence that there is rapid and virtually complete exchange between progesterone and the tritiated steroid at 0 °C within 2 h *in vitro*, we are confident that ^3H -progesterone bound to progesterone receptors in cytosols from the uterus.

The substance R5020 is an interesting tool in studying the mechanism of action of progesterone, but the fact that there are moieties in brain and uterine cytosols which bind progesterone more strongly than they do R5020 militates in favour of the use of progesterone for studying progesterone receptors. In particular, if the corticosteroid-binding globulin-like material (to which R5020 does not bind) is involved in the actions of progesterone and corticosterone in the uterus, there is all the more reason to doubt whether the role of this protein will be elucidated through the use of R5020 alone.

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Polar cap models for pulsars

ARDAVAN¹ has recently claimed on very general grounds that the emission from pulsars must be from the vicinity of the light cylinder. This seems to rule out polar cap models^{2,3} in which the radiation is from particle bunches resulting from a plasma instability near the pulsar. However, the time scale with which the current distribution associated with these bunches fluctuates is much shorter than the pulsar period. A smoothed-out picture of the currents can be stationary in the rotating frame which is a reasonable starting point for theories of the magnetosphere⁴. However, such a smoothing process is unjustified in this context because radiated power varies as the square of a fluctuating current. A simple example is: N electrons moving uniformly round a circle with uncorrelated phases clearly radiate N times what each one does. No matter how large N is, we cannot calculate this radiation using the 'equivalent' steady current. In the present case, the smoothed-out current distribution still has the time dependence caused by the pulsar's rotation and hence radiates mainly from the vicinity of the light cylinder.

Because the fluctuations responsible for the radiation in polar cap models have been smoothed away by Ardaavan¹, the final radiation pattern derived cannot be used to rule out these models.

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ARDAVAN REPLIES—The quasistatic condition¹ sets no restriction on the time scale of fluctuations of the electric current; it merely demands that the dependence of the current on azimuthal angle should be of the same form as its dependence on time. The arbitrary temporal fluctuations of the electric current are thus required to propagate longitudinally so as to create a rigidly rotating pattern. Indeed, unless the time scale with which the current distribution fluctuates is much shorter than the pulsar period, my analysis would predict no radiation in the range of observed frequencies: according to equations (9) and (10) of ref. 1, the power radiated into the higher order harmonics $m \gg 1$ is non-zero only when the Fourier representation of the current density as a function of $\hat{\phi} \equiv \phi - \omega_0 t$ contains the corresponding high-frequency amplitudes.

As long as the interparticle distances within the source do not exceed the wavelength of the radiation, the description based on smoothed-out macroscopic current densities is justified. In the example cited by Nityananda the radiation can in fact be calculated using a macroscopic description; the equivalent quasistatic current density would be

$$j_\phi(r, z, \varphi; t) = \sum_{i=1}^N e\omega_0 \delta(z) \delta(r-r_0) \\ \times \delta(\varphi - \omega_0 t - \varphi_i)$$

where the notation is the same as that of ref. 1. Furthermore, only when $N \rightarrow \infty$ does this current distribution cease to radiate^{2,3}, and then it does so because of the disappearance of all azimuthal fluctuations in the limit and not because of the macroscopic form or the quasistatic character of the current.

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BOOK REVIEWS

Let them eat bread! But which bread?

John Yudkin

DURING the past few years, the consumption of brown bread in Britain has increased more than threefold. It is not hard to see why this is. The Department of Health and the Health Education Council, and similar bodies in many other countries, advocate strongly the consumption of brown and wholemeal breads rather than white, and an increase in the consumption of vegetables and fruit, so as to ensure an adequate intake of dietary fibre. It is now widely accepted that brown is beautiful; and this even extends to an increased consumption of brown sugar and to an unprecedented demand for brown eggs.

Brown eggs, whatever their aesthetic attraction, have no nutritional advantage over white eggs. Brown sugar is better than white only in that a strict avoidance of the latter must lead, for most people, to a sizeable diminution in total sugar consumption. The question then remains whether there is any health benefit in eating brown or wholemeal bread rather than white.

Here are two books that summarize current knowledge, and opinion, of the effects of dietary fibre — the indigestible constituents of cereals, other vegetable foods and fruits which we used to call "roughage". The Plenum book consists of 14 chapters, by 21 different authors, describing in fair detail the research results that have been achieved in studies on the composition of fibre, patterns of consumption of fibre, patterns of consumption within and between different countries, its effects in experimental animals and in human volunteers, and its possible relation to human disease. As in all compilations of this sort, there is some overlap and repetition, as well as rather inadequate consideration of some topics. More interestingly, there are differences in the degree to which different authors are prepared to be hesitant, or enthusiastic, in accepting the largely inconclusive results of experiment as evidence of a role for dietary fibre in the prevention of human disease.

For a careful reading of the evidence shows that dietary fibre, in the quantities found in any ordinary diet, has only three certain effects that are of any consequence to human health. It can speed the passage of the gut contents, and increase the bulk of the faeces; thus, in many individuals, it will relieve constipation. It reduces the symptoms of diverticulitis, although it is often necessary to use more fibre than would normally be present in ordinary

Medical Aspects of Dietary Fibre: A Report of the Royal College of Physicians. Pp.175. ISBN 0-272-79609-3. (Pitman Medical: 1980.) £5.50. *Medical Aspects of Dietary Fiber.* Edited by Gene A. Spiller and Ruth McPherson Kay. Pp.229. ISBN 0-306-40507-5. (Plenum Medical: 1980.) \$32, £20.48.

diets. Third, it reduces the absorption of some of the mineral elements, so that it may lead to deficiency, especially in the elderly.

The Plenum book, then, will be found useful by the large number of people who are nowadays engaged in studying dietary fibre. But the book would also be handy for the nutritionist or the gastroenterologist who is prepared to look in more detail at the justifications for some of the claims that have been made for dietary fibre.

The Royal College of Physicians book, on the other hand, should be read by every dietician, nutritionist and doctor. It was compiled by a committee of the RCP, whose members include some of those who wrote chapters for the Plenum volume. It covers the same ground but in the form of a simply-worded summary of the literature, with conclusions at the end of each chapter. Like the Plenum book, the diseases it considers include obesity, diabetes, ischaemic heart disease, diverticular disease and colon cancer. Here are some of its conclusions:

●Although societies which exist on fibre-rich diet have a low prevalence of obesity, this is not evidence that dietary fibre as such prevents obesity.

●No coherent pattern has emerged of the effects of fibre on the occurrence of ischaemic heart disease. . . . In view of the varying forms of fibre and their variable effects, it is not yet possible to give specific recommendations to the general public about its value in preventing heart attacks.

●Undoubtedly a reduction in the intake of fibre may cause constipation. Nevertheless, other factors may help to explain the commonness of constipation in Westernised communities.

●There are reasonable grounds for the statement that, in genetically susceptible persons, large bowel cancer could be favoured by a fibre-depleted diet, but other explanations for the commonness of this cancer in Westernised countries are possible.

But even this detached assessment of the evidence is occasionally clouded by the current all-pervading atmosphere that

insists that dietary fibre plays an important role in the prevention of disease. The chapter on diabetes ends in this way: "In the long term, replacing refined carbohydrates with physically intact carbohydrate foods might also reduce the risk of diabetes by preventing or arresting obesity". Yet an earlier chapter says: "Dietary fibre has little if anything to do with preventing obesity".

With this careful appraisal of the evidence by such an authoritative body as the RCP, it becomes necessary to ask how it comes about that doctors, health educators, dieticians and nutritionists have almost with one voice been advocating an increase in the consumption of dietary fibre. The two books reviewed have themselves provided much of the answer. It is universally accepted that epidemiological study of populations with different prevalences of a particular diseases may reveal associations but not causes. Yet the major evidence still cited for the view that colonic cancer and several other diseases are caused by a lack of dietary fibre is that rural Africans are much less prone to suffer from these conditions than are urban Westerners. The difference in disease prevalence is indeed likely to be due to differences in environmental conditions, but these differences are many. Some are non-dietary, and include cigarette smoking, physical activity and competitive stress; some are dietary, and include total calories, animal protein, fat, sucrose, calcium and vitamin B₁₂, and not only dietary fibre.

In addition, there is straightforward human prejudice. Part of this derives from people's obsession with their bowels, and accounts for the belief that the many virtues attributed to dietary fibre lie in its ability to cure constipation; in 1979, they spent in the UK more than £6 million in buying non-prescriptive laxatives. Moreover, they are convinced that constipation is in turn the cause of many other and more alarming ills. With the greatest enthusiasm, then, they seized on the suggestion that all this happens because they have been eating white bread.

But the rapid acceptance of the virtues of dietary fibre is not due solely to the hope that it will relieve imaginary maladies. It is also caused — and it is still largely upheld — by the phrase "refined carbohydrate". This refers both to white flour (or bread made from it) and to white sugar. The implication is that they share similar

harmful properties; on the other hand, it is believed that wholemeal flour and raw sugar share similar beneficial properties. But raw sugar is already very much "refined" compared with the sugar cane from which it is made, whereas wholemeal flour really does contain the whole of the wheat berry. Much more importantly, white flour and even pure starch have metabolic properties very different from those of sugar (sucrose).

Denis Burkitt, who, probably more than anyone else, was responsible for the revival of the view that the lack of dietary fibre is the major cause of the ills with which Western countries are plagued, ends his chapter in the Plenum book thus:

The attitude that decries change until a case is proven is both illogical and morally indefensible. Medical history is full of instances in which effective action was taken to reduce the incidence of a disease by avoiding situations known to be associated with it, long before the

cause of the disease was known or the mechanisms involved in its causation understood.

Although they write in a somewhat different context, I much prefer the statement by the authors of another chapter in the same book, as a comment on the too-hasty acceptance of epidemiological evidence unsupported by experiment:

Such an attitude does not seem justified in view of the past continuously preached emphasis on high-fat diet as a plausible risk factor in the etiology of coronary heart disease, which has kept research in preventive cardiology at a standstill for the past 20 years.

Let us by all means eat brown or wholemeal bread if we like it. But let us not delude ourselves that it will make us healthier or prolong our lives. □

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Incomplete expression of genes

Jay Greenberg

Cell Biology: A Comprehensive Treatise. Edited by L. Goldstein and D.M. Prescott. Vol. 3 *Gene Expression: The Production of RNAs*, pp. 684, ISBN 0-12-289503-7. Vol. 4 *Gene Expression: Translation and the Behavior of Proteins*, pp. 496, ISBN 0-12-289504-5. (Academic: 1980.) Vol. 3 \$65, £36.40; Vol. 4 \$49, £27.60.

THESE volumes consist of a collection of articles by different authors on various aspects of gene expression. (Volumes 1 and 2 in the series dealt with cell inheritance and its molecular basis.) They represent a much-needed attempt to fill the gap between reports of original research and the conventional textbook, and they will be of greatest use to students and more advanced scientists who wish to broaden the scope of their knowledge. For the most part, the chapters are not (nor are they intended to be) in-depth, up-to-the-minute reviews for specialists. However, some of them contain detailed, well-referenced information, and they will no doubt be appreciated by experts as well as by less knowledgeable readers.

To a considerable extent these volumes succeed in fulfilling their purpose. However, they are not without shortcomings. Some of the articles are less complete, informative, scholarly and readable than others; furthermore, there is a good deal of overlap between contributions. To some extent, this is the unavoidable result of different authors writing independently of one another; however, the number of redundant passages could have been minimized by more extensive editing.

The chapters are logically organized

around gene expression at the level of RNA transcription and processing (Vol. 3), and translation and post-translational behaviour of proteins (Vol. 4). Unfortunately, however, this arrangement precludes integrated discussions of systems in which gene expression has been investigated at multiple levels. Another problem is that the current rate of progress in cell biology is such that many of the chapters were out of date prior to publication. This is especially true of Vol. 3, which was published late in 1980. However, the references cited in many of the chapters do not go beyond 1978.

Probably the greatest fault (no doubt related to the preceding one) is that neither volume is truly comprehensive. Many aspects of gene expression currently of interest are barely touched. These include such general phenomena as introns, gene splicing and the cytoskeleton, as well as more specific topics such as *Drosophila* heat shock genes, immunoglobulin, globin and ovalbumin genes, and small nuclear RNAs. Control of translation in cell-free systems and virus-infected cells is discussed, as well as regulation of protein synthesis in embryogenesis (masked messengers). However, other phenomena in which translational control plays an important part are not mentioned, for example, mitosis and heat shock. There is little on gene expression in mitochondria and chloroplasts. Apparently, no additional volumes are planned even though the current series is far from complete in its treatment of cell biology. □

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Cosmology at school

Joseph Silk

Physical Cosmology: Les Houches 1979, Session XXXII. Edited by Roger Balian, Jean Audouze and David N. Schramm. Pp.665. ISBN 0-444-85433-9. (North-Holland: 1980.) \$109.75, Dfl.225.

WHAT is the cosmos made of? For all that astronomers can tell, it could be puppy dogs' tails, or back issues of *Nature*, or a host of slightly less exotic possibilities that range from snowballs to giant planets or black holes. All suggestions are equally unproven. What we do know is that the preponderance of matter in the Universe, 90 per cent or more, consists of unknown stuff, for which the term "massonium" was coined at Les Houches by the cosmologist P.J.E. Peebles. Massonium resides in galaxy halos and in the depths of intergalactic space. We infer its presence from its gravitational effect on the observable regions of galaxies. To discover the mystery of massonium is the cosmologists' dream. That it seems destined to remain so is one of the messages that is brought out in *Physical Cosmology*, the report of the 32nd session of the Les Houches Summer School in 1979.

Another elusive cosmological goal is to decide whether the Universe is closed, destined to ultimately collapse to a fiery fate or is open, to expand indefinitely to a cold and ever bleaker future. Here, the evidence presented on the one hand by Peebles and on the other hand by Gustav Tamman, Amos Yahil and Alan Sandage appears to be in conflict. Peebles cites evidence that substantial mass is present in the outer regions of galaxies and galaxy clusters, concluding that within the experimental uncertainties the Universe could be closed. On the other hand, Tamman and his colleagues measure the expansion rate out to a distance from the Milky Way galaxy of some 20 megaparsecs, and find it to be remarkably uniform. This result favours an open Universe, in which the kinetic energy of expansion greatly exceeds the gravitational potential energy that could eventually cause a contraction.

Many of the other topics in *Physical Cosmology*, which span a vast range of astrophysics, are less controversial and provide a good review of the consensus viewpoint towards cosmological issues. As might be anticipated in a lecture course arranged by nuclear astrophysicists Audouze and Schramm, cosmological nucleosynthesis receives a thorough treatment in lectures by Robert Wagoner, Yves David and Hubert Reeves. Helium is the second most abundant element in the Universe, and can be observed in a variety of astronomical objects, from the Sun and nearby HII regions to distant galaxies and remote quasars. Remarkably, astronomers find one nucleus of helium for ten of hydrogen in practically every environment

that can be studied, whereas the abundances of carbon or of iron may vary by one or two orders of magnitude. The big bang theory in its simplest form predicts not just the synthesis of helium when the Universe was one minute old, but even the observed abundance. And this prediction is insensitive to the controversy over whether the Universe is open or closed. Cosmologists have pounced on this coincidence as evidence for the big bang theory itself.

Deuterium is another element that may reflect the history of the early Universe. A by-product of the synthesis of helium, deuterium is also destroyed very effectively, first almost immediately after it forms if the density of the Universe is sufficiently great, and second when stars form. The high temperature achieved in the interior of a star as its nuclear fuel supply of hydrogen burns into helium results in the burning of any pre-existing deuterium. By searching for matter that has not yet undergone this stellar cookery, astronomers have succeeded in detecting deuterium in very young stars and in interstellar matter. The deuterium is believed to have originated along with helium in the big bang; if so, the Universe must be of low density and open in order for a significant quantity to have been synthesized.

Those cosmologists who prefer a dense Universe are unhappy with this result. Fortunately, the discovery announced in 1980 of the possible existence of a mass for the neutrino has managed to satisfy practically everyone. Now, one has the prospect of a dense Universe dominated by neutrinos. At the epoch of nucleosynthesis, the neutrinos play no role, and the Universe behaves as though it had a much lower density, corresponding to that of the protons and producing deuterium in the desired quantity. The massive neutrino promises also to affect many other results in cosmology, especially the work on galaxy formation that is reviewed in the Les Houches lectures by Richard Gott, Brandon Carter and Peter Mészáros.

One of the obvious omissions is the discussion of the pancake theory of galaxy formation. The Moscow school of cosmologists is its most ardent protagonist. Great structures form on the scales of clusters of galaxies, and provide an intriguing picture of the Universe as it appears to be on very large scales, full of sheet-like arrays, filaments and gaping holes. But on the largest scales of all, on the order of and beyond the present horizon, homogeneity is very much apparent, as the lectures of Martin Rees bring out.

One of the joys of *Physical Cosmology* is its illustrations of the lecturers in action. From Wagoner on the grass to Rees at the blackboard, these candid shots add a dash of vitality that provides an extra reward for those brave souls who have mortgaged their possessions to buy this book. □

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Sites of special spiritual importance

Kenneth Mellanby

The IBP Survey of Conservation Sites: An Experimental Study. IBP, 24. Edited by A.R. Clapham. Pp.344. ISBN 0-521-22697-X. (Cambridge University Press: 1980.) £25, \$57.50.

ONLY within the past hundred years have there been attempts to conserve wildlife for its own sake, and to establish nature reserves in which plants and animals can continue to exist under something approaching natural conditions. It has not always been easy to decide upon what criteria nature reserves, national parks and other protected areas should be selected. In the early days we relied on the instincts of a few dedicated naturalists. Today, many would like to use more scientific methods.

The International Biological Programme (IBP) was established in 1964, to try to obtain a better understanding of the environment as a basis for rational management of natural resources. Much of its work concerned productivity and potential food production in a world with a growing population. Its Terrestrial Conservation Section (CT) decided "to initiate a world-wide study of the great variety of terrestrial ecosystems, leading to the selection of representative samples of all the more important ones". This book contains the results of the survey.

The method used to evaluate sites was the IBP check-sheet survey. 13,000 copies of the check-sheet, details of which are given in a 22-page appendix, were distributed world-wide. 3,010 were returned, 2,690 satisfactorily completed. Most returns came from the developed countries, Britain, the USA, Europe (where Poland excelled), Japan and Australia. These results may seem disappointing, but they gave much more information than had previously been available on a world scale. The book describes how the information was processed, and includes critical discussions of the methodology. It should be of great value to those concerned with the survey and classification of vegetation on an international and a national scale. With its

appendices dealing with different techniques for vegetational classification it will be a useful tool for others involved in this field, though it can hardly be recommended as a good read even for the most dedicated ecologist.

No one can doubt the scientific value of this study, as an academic exercise in ecology. Its value in the selection of potential nature reserves is more doubtful. In the past most reserves have been established because the area has appealed to the emotions of the selector, and because it has been available. The process has been essentially hit or miss — it may be surprising that there have been so many hits, and that so many of the older reserves are still obviously of considerable value. There are many who still consider that this is the best method of selection, particularly in Britain where most of the treasured sites have been profoundly altered by human activities. Many of the scientific reasons for nature conservation, such as maintenance of a large heterogeneous gene-pool or the perpetuation of the full diversity of the world's plant and animal communities, have been described as rationalizations of decisions made for aesthetic and emotional reasons. It has even been suggested that Sites of Special Scientific Interest might be better designated Sites of Special Spiritual Importance.

I believe that these subjective emotions are often the reason why many of us are interested in wildlife conservation. However, this does not mean that a scientific study of the results of emotions is not still valuable. So this exercise is to be welcomed as giving us a clearer picture of the world scene, and if its application means that more unspoiled areas can be preserved for future generations we should all be grateful for those who completed what, to me, would have been a very tedious operation. □

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Polyhedra for chemists (and aesthetes)

Michael Mingos

Transition Metal Clusters. Edited by B.F.G. Johnson. Pp.681. ISBN 0-471-27817-3. (Wiley: 1980.) £33, \$88.15.

THE scientific interest in polyhedra can be traced back to ancient Greece, and the understanding of the mathematical properties of polyhedra developed then and subsequently have often proved to be

relevant to physics and chemistry. With this long tradition, therefore, it is not surprising that during the past 20 years transition metal chemists have been particularly interested in synthesizing new "cluster" compounds which have between four and forty metal atoms bonded together in a wide variety of polyhedral arrangements. Aside from the aesthetic

satisfaction of elucidating the structures of these beautiful molecules, such compounds have provided a range of interesting theoretical valency problems and have stretched to the limits the techniques currently available to chemists for characterizing molecules. In addition, they have stimulated the interest of industrial chemists and molecular biologists: the former because of the potential of the compounds as homogeneous and heterogeneous catalysts, and the latter because iron sulphur clusters have been found to be important components of ferredoxin and nitrogenase.

Transition Metal Clusters therefore represents a timely attempt to bring together under one cover some of the developments in this area.

The book covers most of the important aspects of cluster compounds, with P.R. Raithby reviewing the geometries of more than 1,000 cluster compounds whose structures have been elucidated using X-ray and neutron diffraction techniques. K. Wade and R.G. Wooley have contributed two very different views of the bonding in these polyhedral molecules, J.A. Connor has provided a balanced and critical account of the thermochemical estimation of metal-metal bond strengths in cluster compounds, and B.F.G. Johnson and R.E. Benfield have discussed their fluxional characteristics. The bio-

logical significance of cluster compounds is stressed by C.D. Garner, in a chapter dealing with cubane clusters, and R. Whyman has attempted to evaluate whether cluster compounds do actually function as effective catalysts by reviewing the relevant academic and patent literature. There is also a detailed review by A.J. Deeming on the reactions of metal carbonyl cluster compounds.

The book gains by drawing on the expertise of the authors to provide a reasonably up-to-date (literature references up to the end of 1978) and broad view of the field, but suffers from an uneven view of the potential readership of the book. Thus although some chapters are written at a level which would be appreciated by a third-year undergraduate, others are in the style of a research paper or a terse contribution to a Chemical Society *Specialist Periodical Report*. Therefore, although I wholeheartedly agree with the editor that this book will serve as a "source of reference to workers in the field", I very much doubt if it will be used much as "an advanced text for undergraduates or graduates in chemistry". □

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into a battery cage, but this does not reduce the suffering of those already in occupation. In fact, reading these chapters it becomes clear that there is no single way in which the suffering of an animal can be clearly quantified. Ideally, it would be nice to be able to measure some physiological or biochemical variable which could be used as an index of "stress". Unfortunately, although certain parameters can be measured which give an indication of the state of the general adaptation syndrome, there is no evidence that a complete absence of "stress" (which can itself be defined in many different ways) is necessarily beneficial to an animal. Nor can we say that an animal suffers because it is forced to lead an "unnatural" life. In nature many animals have a relatively short life-span, quickly succumbing to predators, disease, exposure or starvation. Their death may be lingering and painful. Even allowing animals to choose the conditions under which they will live does not always help. Animals sometimes choose a course of action which is not in their best interests; few domestic pets enjoy a visit to the vet, even though this is usually for their benefit.

Does this mean that we are unable to assess animal suffering at all? Dr Dawkins is clearly concerned that the general tone of these early chapters "may have seemed rather negative", and she goes on to provide a list of questions which we must ask ourselves in order to determine whether an animal is suffering unduly. We must consider the conditions in which the animals are kept, whether they are healthy, whether they appear physiologically or behaviourally abnormal, and if so what is likely to be the cause of these abnormalities. If possible we should also determine what conditions the animals themselves prefer. And in the end, the assessment will have to be subjective. "There is no formula for giving a clear yes/no answer to the question of whether an animal is suffering". Even if we could answer the question unequivocally, we would still have to decide how much suffering we should permit in relation to the benefits we obtain from animals. That, however, is a whole new subject which falls more in the field of ethics than biology.

Dr Dawkins has produced an excellent review of the state of our ignorance about suffering in animals, and has highlighted many inconsistencies in our attitudes. This book should be read by all those involved with farm and laboratory animals, in the hope that they will endeavour to reduce needless suffering wherever it occurs. If we must cause suffering to animals in the name of agriculture or medical research, let us at least make sure that it is not caused unnecessarily through our ignorance of the needs of those creatures that provide us with so many benefits. □

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Animals: their use and their abuse

Michael F.W. Festing

Animal Suffering: The Science of Animal Welfare. By Marian Stamp Dawkins. Pp.149. Hbk ISBN 0-412-22580-8; pbk ISBN 0-412-22590-5. (Chapman & Hall: 1980.) Hbk £7.50; pbk £3.95.

ON AVERAGE, a person living in a developed country such as the UK will be responsible for the death of about 600 chickens, 22 pigs, 20 sheep and 7 cows for food and 5 mice, 2 rats, 1 chicken, a third of a guinea-pig and two-hundredths of a cat and dog for medical research during their 75-year life span. They will also be responsible for the deaths of countless fish, both for food and for sport. Their food supplies and health will also be maintained at the cost of the death by poison or trapping of an unknown, but large number of mammals and birds classified as pests. Who can doubt that the production, maintenance and killing of all these animals involves a certain amount of suffering? The problem for us as individuals, and as a society, is to decide what to do about it, if anything.

In order to make some progress, we must sort out our moral attitudes to animals and to our fellow human beings, and we must

attempt to strike a balance between the amount of suffering which we cause and the benefits which we derive from the animals. This is an extremely difficult problem, because it is almost impossible to quantify either the benefits of, say, using animals in medical research, or the amount of suffering that we cause. This book is an attempt to summarize our knowledge about suffering in animals, and also point out many inconsistencies in our attitudes to other species.

Following an introductory chapter, the assessment of animal suffering is considered from several different points of view. These include suffering and health, "productivity", the "unnatural" life, the physiology of suffering, behaviour, what animals choose and analogies with ourselves.

Physical health is seen to be a necessary, but certainly not a sufficient condition for freedom from suffering, and is probably the single most useful criterion of animal welfare. Productivity, in the sense used in agriculture, is more concerned with economics than animal welfare. A higher productivity (i.e. profit) may well be achieved by putting an additional chicken